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Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

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HYGIENIC LABORATORY.—BULLETIN No. 29.

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M. J. ROSENAU, Director.

APRIL, 1906.

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A STUDY OF THE CAUSE OF SUDDEN DEATH FOLLOWING THE INJECTION OF HORSE SERUM.

BY

M. J. ROSENAU

AND

JOHN F. ANDERSON.

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The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

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No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

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No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

(Continued on third page of cover.)

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A STUDY OF THE CAUSE OF SUDDEN DEATH FOLLOWING THE INJECTION OF HORSE SERUM.

By Milton J. Rosenau,
Passed Assistant Surgeon, Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.
and
John F. Anderson,
Passed Assistant Surgeon, Assistant Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

It has long been known that the blood of certain animals is poisonous when transfused or injected into certain other species. Many instances might be cited showing that the blood serum of one animal has poisonous properties when injected into an animal of another species. But the blood serum of the horse apparently lacks such poisonous action. Very large quantities of the blood serum of the horse may be injected into man, rabbits, guinea pigs, and many other animals without serious inconvenience, except occasionally a slight reaction at the site of inoculation.

In a certain proportion of cases the injection of horse serum into man is followed by urticarial eruptions, joint pains, fever, swelling of the lymph nodes, edema, and albuminuria. This reaction, which appears after an incubation period of eight to thirteen days, has been termed by Pirquet and Schick the "serum disease."

In exceptional instances sudden death has followed an injection of horse serum in man.

These studies were taken up in October, 1905, in order to throw light upon the cause of this unfortunate accident. We have shown that ordinarily horse serum is a comparatively bland and harmless substance when injected into certain animals: but these animals may be rendered so susceptible that an injection of horse serum may produce sudden death or severe symptoms. For example, large quantities of horse serum may be injected subcutaneously or into the peritoneal cavity of a guinea pig without apparently causing the animal the least inconvenience. However, if a guinea pig is injected with a small quantity, say $\frac{1}{250}$ c. c., of horse serum and after the expiration of a certain interval is again injected with horse serum the result will probably be fatal. The first injection of horse serum has sensitized the animal in such a way as to render it very susceptible to a toxic principle in horse serum. It is probable that when the guinea pig is injected with the first, or sensitizing, quantity of serum the strange proteid contained in the horse serum develops in the body of the guinea pig "antibodies" which, when brought into contact with more horse serum given at a second injection, produce either a union or a reaction, which causes the toxic action.

A certain time is necessary to elapse between the first and second injections of horse serum before this toxic action is able to manifest itself. This "period of incubation" is from ten to twelve days, and corresponds suggestively with the period of incubation of the serum disease which Pirquet and Schick place at eight to thirteen days.

Guinea pigs may be sensitized with exceedingly small quantities of horse serum. In most of our work we used quantities less than $\frac{1}{250}$ c. c. and we found in one instance that $\frac{1}{1,000,000}$ c. c. of horse serum was sufficient to render a guinea pig susceptible.

It also requires very small quantities of horse serum, when given in a second injection, to produce poisonous symptoms. One-tenth c. c. injected into the peritoneal cavity is sufficient to cause the death of a half-grown guinea pig. One-tenth c. c. of horse serum injected subcutaneously is sufficient to produce serious symptoms. The fact that this toxic action may be developed by such small quantities of serum and the fact that exceedingly small quantities are sufficient to produce symptoms and death upon a second injection, a priori places both the sensitizing and the toxic principle in the horse serum in the "haptin group" of substances in the sense used by Ehrlich.

A still further indication that the side-chain theory in its broadest sense may be applicable is the further fact that immunity may be produced against the toxic action by multiple injections of the serum.

While at first we thought that diphtheria antitoxin had some relation to this action, we are now able to state positively that it has nothing whatever to do with the poisonous action of horse serum; further, that diphtheria antitoxin in itself is absolutely harmless. The toxic action which we have studied is caused by a principle in normal horse serum and is entirely independent of the antitoxic properties of the serum.

Part I.

CONTROL EXPERIMENTS.

ACTION OF NORMAL HORSE SERUM UPON NORMAL GUINEA PIGS.

Normal horse serum when injected into normal guinea pigs causes no symptoms. Large amounts, such as 6 or 10 c. c., may be injected into the peritoneal cavity of a guinea pig without any apparent inconvenience to the animal. When normal horse serum is injected subcutaneously into the guinea pig it is sometimes either absorbed very slowly or there is a slight local reaction, as indicated by edema and induration of the subcutaneous tissue at the site of injection.

G. P. No. 2.[a] Six c. c. normal horse serum (roan horse) injected into the peritoneal cavity. No symptoms.

G. P. No. 3. Ten c. c. normal horse serum (roan horse) injected into the peritoneal cavity. No symptoms.

G. P. No. 63. Six c. c. normal horse serum (Sp. horse) injected into the peritoneal cavity. No symptoms.

G. P. No. 64. Six c. c. normal horse serum (Jane horse) injected into the peritoneal cavity. No symptoms.

G. P. No. 66. Six c. c. normal horse serum (Sam horse) injected into the peritoneal cavity. No symptoms.

G. P. No. 65. Six c. c. normal donkey serum (donkey No. 58) injected into the peritoneal cavity. No symptoms.

G. P. No. 296. Six c. c. normal donkey serum injected into the peritoneal cavity. No symptoms.

G. P. No. 297. Do.

G. P. No. 298. Six c. c. normal horse serum (Sp.) injected into the peritoneal cavity. No symptoms.

G. P. No. 299. Do.

Specimens of serum from each one of the normal horses used in this work appear in the above series of guinea pigs, which serve as controls.

Donkey serum is also harmless for normal guinea pigs and is just as toxic as horse serum to a sensitized guinea pig.

Uhlenhuth (Zur Kenntniss der giftigen Eigenschaften des Blutserums, Ztschr. f. Hyg., v. 26, 1897, p. 384) found that normal horse serum, when injected intravenously into rabbits, is harmless. He injected as large quantities as 60 c. c. per 1 kilogram of rabbit and found that this caused no reaction at all. On the other hand he found that 11 c. c. of sheep serum, 12 c. c. of hog serum, or 6 c. c. of cattle serum per 1 kilogram of rabbit, when injected intravenously, caused the death of the animal.

The nontoxic action of horse serum on animals was further shown by Hermann Pfeiffer (Ueber die nekrotisirende Wirkung normaler

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[a] G. P., abbreviation for guinea pig. The numbers refer to the laboratory serial numbers.

Seren. Ztschr. f. Hyg. u. Infectionskrankh., v. 51, (2), pp. 181–196, 1905), who first confirmed the above work of Uhlenhuth and then showed that the subcutaneous inoculation of large quantities of horse serum into guinea pigs produced at most a slight local reaction. He found on the other hand that the serum of certain other animals, e. g., cattle, hog, man, when injected subcutaneously into guinea pigs, produced a local reaction and in large quantities caused necrosis.

We took the temperature of a number of guinea pigs twice daily for eighteen days following the injection of large quantities of horse serum subcutaneously, in order to determine whether a febrile reaction followed. No marked deviation from the normal temperature was noted.

ACTION OF ANTITOXIC HORSE SERUM UPON NORMAL GUINEA PIGS.

It is a well-known fact that large quantities of horse serum containing high antitoxic potency may be injected subcutaneously or into the peritoneal cavity of a normal guinea pig without causing symptoms. We use this method of testing the diphtheria antitoxin bought upon the open market to determine its freedom from bacterial and toxic impurities. Of the many guinea pigs thus inoculated none has shown untoward effects except in the case of bacterial or toxic contaminations. This method of testing the purity of antitoxin is also used by the manufacturers almost daily. We may therefore state that diphtheria antitoxic serum when injected into a normal guinea pig causes no symptoms.

However, we present the following experimental data, which serve as controls for this work, because the normal guinea pigs in the following series have been inoculated into the peritoneal cavity with large quantities of the antitoxic horse serums subsequently used in this work.

G. P. No. 300. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. No symptoms.

G. P. No. 301. Do.

G. P. No. 302. Six c. c. antitoxic horse serum (Natl. V. 14) injected into the peritoneal cavity. No symptoms.

G. P. No. 303. Do.

G. P. No. 304. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity.
No symptoms.

G. P. No. 305. Do.

G. P. No. 306. Six c. c. antitoxic horse serum (NYBH 10B, Gibson) injected into the peritoneal cavity. No symptoms.

G. P. No. 307. Six c. c. antitoxic horse serum (NYBH 13C, Gibson) injected into the peritoneal cavity. No symptoms.

G. P. No. 308. Six c. c. antitoxic horse serum (Alex. A 211) injected into the peritoneal cavity. No symptoms.

G. P. No. 309. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

G. F. No. 310. Do.

Part II.

HORSE SERUM IS POISONOUS TO A "TREATED" OR "USED" GUINEA PIG.

It has long been known by some of the manufacturers of diphtheria antitoxin that when antidiphtheric serum is inoculated into a used guinea pig the result is almost invariably death. By a "used" or "treated" guinea pig we mean one that has recovered from the effects of an inoculation of the toxine-antitoxin mixture used in testing the potency of diphtheria antitoxin. For example:

G. P. No. 4191. Six c. c. antitoxic horse serum (Alex. 192) injected into the peritoneal cavity. Death in 15 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{380}$ c. c. antitoxic horse serum (PD&Co 08033).]

G. P. No. 4103. Nine c. c. antitoxic horse serum (Alex. 193) injected into the peritoneal cavity. Dead in 5 minutes.

[Previous treatment: 31 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 4330. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into peritoneal cavity. Dead in 2 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{200}$ c. c. antitoxic horse serum (Alex. 185).]

G. P. No. 6B. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into peritoneal cavity. Dead in 27 minutes.

[Previous treatment: 86 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B25).]

G. P. No. 4381. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 11 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{310}$ c. c. antitoxic horse serum (Natl. V. 10).]

G. P. No. 4336. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 12 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{360}$ c. c. antitoxic horse serum (PD&Co 07635).]

G. P. No. 4369. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{400}$ antitoxic horse serum (Cutter 1461).]

G. P. No. 4377. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. antitoxic horse serum (Natl. V. 7).]

G. P. No. 4526. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 30 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4491. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 41 minutes.

[Previous treatment: 18 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4494. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 37 minutes.

[Previous treatment: 19 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 75. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 3 hours.

[Previous treatment: 15 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{250}$ c. c. antitoxic horse serum (Natl. IX. 17).]

G. P. No. 88. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 15 days prior, inoculated subcutaneously with 0.006 c. c. toxine No. 7+1 unit antitoxic horse serum (Natl. IX. 17).]

G. P. No. 4522. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 20 minutes.

[Previous treatment: 20 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4500. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4471. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 8 minutes.

[Previous treatment: 22 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{250}$ c. c. antitoxic horse serum (Natl. IX. 17).]

G. P. No. 4503. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.[a]

[Previous treatment: 22 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4499. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 20 minutes.

[Previous treatment: 24 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4465. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 40 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{250}$ c. c. antitoxic horse serum (Natl. VIII. 17).]

G. P. No. 4387. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.2 c. c. toxine No. 58+1 unit antitoxic horse serum (Standard B27).]

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[a] Explanation of occasional irregularities, page 63.

G. P. No. 4509. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 143. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 73. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 57 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+250 units antitoxic horse serum (Natl. IX. 17).]

G. P. No. 82. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 57 days prior, inoculated subcutaneously with 0.006 c. c. toxine No. 7+$\frac{1}{250}$ c. c. antitoxic horse serum (Natl. IX. 17).]

G. P. No. 139. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 49 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 1351).]

All of the above pigs were first given the toxine-antitoxin mixtures in order to test the strength of diphtheria antitoxic serum bought on the open market in accordance with the law of July 1, 1902.

Horse serum is also toxic when injected subcutaneously and in small quantities, as will be seen from the following experiments:

G. P. No. 4951. Six c. c. normal horse serum (roan) inoculated subcutaneously. Dead in 35 minutes.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{350}$ c. c. antitoxic horse serum (NYBH 10B).]

G. P. No. 5047. Same injection. Dead in 40 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{400}$ c. c. antitoxic horse serum (Alex. A205).]

G. P. No. 4950. Same injection. Symptoms; recovered.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{300}$ c. c. antitoxic horse serum (NYBH 10B).]

G. P. No. 5046. Same injection. Dead in 40 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{400}$ c. c. antitoxic horse serum (Alex. A204).]

G. P. No. 4524. Six c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{350}$ c. c. antitoxic horse serum (Alex. 192).]

G. P. No. 4623. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{300}$ c. c. antitoxic horse serum (Alex. 192).]

G. P. No. 4512. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Dead in 81 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4502. Six c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4514. Two-tenths c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

SYMPTOMS CAUSED BY THE INJECTION OF HORSE SERUM INTO A SUSCEPTIBLE GUINEA PIG.

Very characteristic symptoms are produced by horse serum, either normal or antitoxic, when injected into a susceptible guinea pig. The symptoms are apparently the same whether the injection is made subcutaneously or into the peritoneum, or whether normal or antitoxic horse serum is used. In five or ten minutes after injection the pig manifests indications of respiratory embarrassment by scratching at the mouth, coughing, and sometimes by spasmodic, rapid, or irregular breathing; the pig becomes restless and agitated. This stage of exhilaration is soon followed by one of paresis or complete paralysis. The pig is unable to stand or, if it attempts to move, falls upon its side; when taken up it is limp. Spasmodic, jerky, and convulsive movements now supervene.

Pigs in this stage with complete paralysis may fully recover, but usually convulsions appear, and are almost invariably a forerunner of death. Symptoms appear about ten minutes after the injection has been given; occasionally in pigs not very susceptible they are delayed thirty to forty-five minutes. Only in one or two instances of the several hundred pigs which we have observed have the symptoms developed after one hour. Pigs developing symptoms as late as this are not very susceptible and do not die. The chain of symptoms is exceedingly characteristic. The symptoms do not always follow in the order given. Death usually occurs within an hour and frequently in less than thirty minutes.

THE POISONOUS ACTION OF HORSE SERUM ACTS UPON THE RESPIRATORY CENTERS.

Judging from the symptoms produced by the injection of horse serum into a susceptible guinea pig we assumed that the poison acted upon the nervous system. Autopsies done immediately after the death of the guinea pigs showed invariably that the heart continued to beat long after respiration had ceased. In some instances the heart would continue to beat a full hour when exposed. This would seem to indicate that we were dealing with a poison which caused death through the nervous control of the respiration, and the following experiment shows that this effect is certainly not local.

G. P. No. 4526. Six c. c. antitoxic horse serum (National VIII. 17) were injected into the peritoneal cavity. Death in 30 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with 0.19 c. c. of toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

Immediately after death the phrenic nerve was exposed and stimulated high up, causing contractions of the diaphragm both upon making and breaking the galvanic current. The contractions were also caused with the induced current. The contractions of the diaphragm caused by stimulating the phrenic nerve in this way were produced with a weaker current than those required to cause similar contractions in a normal control guinea pig.

Part III.

THE TOXIC ACTION BEARS NO RELATION TO DIPHTHERIA.

THE POISON IS NOT TOXONE.

It occurred to us that possibly diphtheria antitoxic serum may contain free toxone which, when injected into a "used" or "treated" guinea pig, might produce acute effects upon the nervous system of such a pig, which, on account of the previous effects of diphtheria toxine, had been rendered peculiarly susceptible. It is well known that the toxone does not cause the acute death of a guinea pig, but only produces a local edema and late paralysis. This fact, first demonstrated by Ehrlich, may readily be demonstrated by inoculating guinea pigs with toxine and antitoxin mixed in such proportions so that the toxine is but partly neutralized. We were further led to believe that the poisonous effects of horse serum upon a treated guinea pig might be a manifestation of the toxones because the symptoms so plainly point to the nervous system. The pigs almost invariably show paresis or paralysis.

The fact that neither toxone nor any other constituent in the toxine plays any rôle in the poisonous action of horse serum is demonstrated in the following experiment:

G. P. No. 4161. 0.1 c. c. toxine No. 15 (MLD=.01) was injected into the peritoneal cavity. No symptoms. Completely paralyzed at the time, resulting from previous treatment.

[Previous treatment: 31 days previously inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{210}$ c. c. diphtheria antitoxic serum (Mulford's No. 942).]

One hour 50 min. later this same pig was inoculated with 6 c. c. diphtheria antitoxic serum (P. D. & Co. No. 08022) into the peritoneal cavity. Death in 10 minutes.

It is well known that overpowering doses of toxine injected either subcutaneously or into the peritoneal cavity of a guinea pig require about twenty hours to kill the animal. These large doses of toxine do not cause immediate symptoms.

It occurred to us, however, that while toxine alone may not have this power, nevertheless when it is mixed with antitoxic horse serum and when large doses of the mixture are injected into a guinea pig there may be a sufficient liberation of toxone or other poisonous substances capable of producing symptoms and sudden death.

The following mixture of toxine and antitoxin represents these two substances in the proportion of the L° dose:

G. P. 4483. 1.6 c. c. toxine No. 7 + 11.5 units antitoxic serum (standard B27) injected subcutaneously. No symptoms.

18 days later 6 c. c. antitoxic horse serum (National VIII, 17) injected into the peritoneal cavity of the same pig. Death 30 minutes.

In a similar way the effects of mixing large doses of the diphtheria toxine with normal horse serum were tested, as follows:

G. P. No. 40. Six c. c. normal horse serum (roan horse) + 0.3 c. c. diphtheria toxine No. 5 mixed in vitro and injected into the peritoneal cavity. No immediate symptoms. Died 2 days later.

GUINEA PIGS CAN NOT BE RENDERED SUSCEPTIBLE BY PRIOR INFECTIONS WITH THE BACILLUS DIPHTHERIÆ.

When this work was first undertaken it was thought to have a bearing upon sudden deaths in children suffering with diphtheria. The work was begun in order to discover, if possible, the cause of this accident. We thought that possibly by injecting small quantities of a virulent culture of the Klebs-Löffler bacillus into guinea pigs, in that way imitating mild cases of diphtheria, some of these pigs could be rendered susceptible to subsequent inoculations of antitoxic horse serum. This, however, is not the case for guinea pigs, as is plainly seen in the following experiments, in which a number of guinea pigs were treated with sufficient quantities of a culture of the Klebs-Löffler bacillus to cause slight symptoms and at varying intervals of time were inoculated again into the peritoneal cavity with large quantities of antitoxic horse serum. In no instance did the guinea pigs show any ill effects from this latter treatment.

G. P. No. 41. Six c. c. antitoxic horse serum (Natl. VIII, 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment. *4 days prior*, inoculated subcutaneously with 0.1 c. c. suspension.]

G. P. No 9. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: *7 days prior*, inoculated subcutaneously with 1 gtt. of 24-hour suspension.]

G. P. No. 51. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: *7 days prior*, inoculated subcutaneously with 0.1 c. c. of suspension.

G. P. No. 42. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: *9 days prior*, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

G. P. No. 56. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: *9 days prior*, inoculated subcutaneously with 0.1 c. c. suspension.]

G. P. No. 43. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: *12 days prior*, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

G. P. No. 45. Six c. c. same serum and injection as g. p. 41. No symptoms.

[Previous treatment: *14 days prior*, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

G. P. No. 58. Six c. c. same serum and injection as g. p. 41. No symptoms.
[Previous treatment: *21 days prior*, inoculated subcutaneously with 0.1 c. c. suspension.]

G. P. No. 44. Six c. c. same serum and injection as g. p. 41. No symptoms.
[Previous treatment: *24 days prior*, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

G. P. No. 52. Six c. c. same serum and injection as g. p. 41. No symptoms.
[Previous treatment: *27 days prior*, inoculated subcutaneously with 0.1 c. c. 24-hour suspension.]

G. P. No. 50. Six c. c. same serum and injection as g. p. 41. No symptoms.
[Previous treatment: *32 days prior*, inoculated subcutaneously with 1 gtt. 24-hour suspension.]

DIPHTHERIA TOXINE CAN NOT RENDER GUINEA PIGS SUSCEPTIBLE.

For the same reason that guinea pigs were treated with cultures of the *Bacillus diphtheriæ* we also attempted to render them susceptible by the injection of small quantities of diphtheria toxine. On account of the great importance of this subject a large number of guinea pigs were used to demonstrate the rôle which the diphtheria toxine may play in the subsequent action of horse serum. Our experiments demonstrate conclusively that diphtheria toxine alone plays absolutely no rôle in rendering guinea pigs susceptible to the toxic action of horse serum; for in no instance, as will be seen in the following series, does the guinea pig show the slightest effects when subsequently injected with large quantities of serum.

G. P. No. 4584. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.
[Previous treatment: *2 days prior*, inoculated subcutaneously with 0.001 c. c. toxine No. 7.]

G. P. No. 4593. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *3 days prior*, as g. p. 4584.]

G. P. No. 4534. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *4 days prior*, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]

G. P. No. 4550. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *5 days prior*, as g. p. 4534.]

G. P. No. 4548. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *6 days prior*, as g. p. 4534.]

G. P. No. 4585. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *6 days prior*, as g. p. 4584.]

G. P. No. 4432. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *7 days prior*, as g. p. 4534.]

G. P. No. 4434. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *7 days prior*, inoculated subcutaneously with 0.004 c. c. toxine No. 7.]

G. P. No. 4426. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *8 days prior*, as g. p. 4534.]

G. P. No. 4583. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *9 days prior*, as g. p. 4534.]

G. P. No. 4579. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *10 days prior*, as g. p. 4534.]

G. P. No. 4582. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *11 days prior*, as g. p. 4534.]

G. P. No. 4430. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *12 days prior*, as g. p. 4534.]

G. P. No. 4572. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *12 days prior*, as g. p. 4534.]

G. P. No. 4568. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *13 days prior*, as g. p. 4534.]

G. P. No. 4576. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *14 days prior*, as g. p. 4534.]

G. P. No. 4577. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *16 days prior*, as g. p. 4534.]

G. P. No. 4535. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *17 days prior*, as g. p. 4534.]

G. P. No. 4547. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *18 days prior*, as g. p. 4534.]

G. P. No. 4580. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *19 days prior*, as g. p. 4534.]

G. P. No. 4556. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *20 days prior*, as g. p. 4534.]

G. P. No. 4558. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *21 days prior*, as g. p. 4534.]

G. P. No. 4545. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *23 days prior*, as g. p. 4534.]

G. P. No. 4555. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *24 days prior*, as g. p. 4534.]

G. P. No. 4541. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *26 days prior*, as g. p. 4534.]

G. P. No. 4404. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *27 days prior*, inoculated subcutaneously with 0.002 c. c. toxine No. 5.]

G. P. No. 4454. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *27 days prior*, inoculated subcutaneously with 0.01 c. c. toxine No. 19.]

G. P. No. 4565. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *27 days prior*, as g. p. 4534.]

G. P. No. 4554. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *30 days prior*, as g. p. 4534.]

G. P. No. 4578. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *31 days prior*, as g. p. 4534.]

G. P. No. 4542. Six c. c. same serum and injection as g. p. 4584. No symptoms.
[Previous treatment: *33 days prior*, as g. p. 4534.]

G. P. No. 4529. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.
[Previous treatment: *46 days prior*, as g. p. 4534.]

G. P. No. 4428. Do.

G. P. No. 4427. Do.

G. P. No. 4431. Do.

G. P. No. 4567. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.

[Previous treatment: *65 days prior,* as g. p. 4534.]

G. P. No. 4554. Do.

G. P. No. 4553. Do.

DIPHTHERIA ANTITOXIN PLAYS NO RÔLE IN THIS ACTION.

Before we realized that the diphtheria toxine or antitoxin played no rôle in rendering guinea pigs susceptible to subsequent injections of horse serum we prepared the following series of guinea pigs, in order to eliminate the antitoxin as a factor. These pigs were injected subcutaneously with a mixture of 1 c. c. of normal horse serum and a small quantity of diphtheria toxine, slightly less than a minimal lethal dose. This was done for comparison with the susceptibility produced by the toxine-antitoxin mixtures.

G. P. No. 91. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms. Died 12 hours later.

[Previous treatment: 65 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 296. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 60 minutes.

[Previous treatment: 71 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 96. Do. Dead in 40 minutes.

G. P. No. 93. Do. Symptoms; recovered.

G. P. No. 90. Do.

It will be seen from our experiments that guinea pigs are quite as sensitive when previously treated with toxine-normal serum as with the toxine-antitoxin mixtures.

In order to compare the toxic action of normal serum with antitoxic serum upon pigs so treated, four animals of the above series were inoculated with antitoxic horse serum, as follows:

G. P. No. 92. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms. Died 12 hours later.

[Previous treatment: 15 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 98. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 53 minutes.

[Previous treatment: 58 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

G. P. No. 95. Do. Dead in 43 minutes.

G. P. No. 94. Same injection. Symptoms; recovered.

[Previous treatment: 65 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7+1 c. c. normal horse serum (Sam).]

It is evident from the above that fresh normal serum is quite as poisonous to a sensitized guinea pig as is antitoxic horse serum.

In order further to eliminate the antitoxin as a factor in this problem, the following series of used or treated guinea pigs were injected with normal horse serum:

G. P. No. 4100. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 36 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{250}$ c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 4105. Same injection. Dead in 30 minutes.

[Previous treatment: 36 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Alex. 189).]

G. P. No. 4106. Same injection. Dead in 5 minutes.

[Previous treatment: 36 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{360}$ c. c. antitoxic horse serum (Alex. 189).]

G. P. No. 4331. Same injection. Dead in 90 minutes.

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{250}$ c. c. antitoxic horse serum (Alex. 185).]

G. P. No. 4351. Same injection. Dead in 40 minutes.

[Previous treatment: 18 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{460}$ c. c. antitoxic horse serum (PD&Co 08021).]

G. P. No. 3950. Six c. c. normal horse serum (Sam) injected into peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{280}$ c. c. antitoxic horse serum (Hubbert 1).]

G. P. No. 3951. Six c. c. normal horse serum (Jane) injected into the peritoneal cavity. Dead in 30 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{290}$ c. c. antitoxic horse serum (Hubbert 1).]

G. P. No. 3952. Six c. c. normal horse serum (Sp.) injected into the peritoneal cavity. Dead in 38 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{290}$ c. c. antitoxic horse serum (Hubbert 1).]

G. P. No. 3953. Six c. c. normal donkey serum (No. 58) injected into the peritoneal cavity. Dead in 60 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{310}$ c. c. antitoxic horse serum (Hubbert 1).]

G. P. No. 4109 One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+ $\frac{1}{440}$c. c. antitoxic horse serum (Alex. 188).]

G. P. No. 4945. Six c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{420}$ c. c. antitoxic horse serum (Wellcome 478).]

G. P. No. 4934. Six c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{350}$ c. c. antitoxic horse serum (Wellcome 479).]

G. P. No. 4930. Six c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{300}$ c. c. antitoxic horse serum (Wellcome 475).]

G. P. No. 4931. Six c. c. normal horse serum (roan) inoculated subcutaneously. Dead in 5 minutes.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{370}$ c. c. antitoxic horse serum (Wellcome 475).]

G. P. No. 4941. Six c. c. normal horse serum (roan) inoculated subcutaneously. Dead in 20 minutes.

[Previous treatment: 34 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{700}$ c. c. antitoxic horse serum (Wellcome 474).]

All of the above animals and most of those which appear upon the subsequent pages of this bulletin were first used to test the strength of the diphtheria antitoxin bought on the open market in accordance with the law of July 1, 1902.

The following series of guinea pigs were given small quantities of antitoxic horse serum, some subcutaneously and some intraperitoneally, in order to compare the sensitizing action by these two methods of inoculation. At the second injection they were all given 6 c. c. of normal horse serum into the peritoneal cavity.

G. P. No. 276. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 25 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. antitoxic horse serum (Natl. V. 14).]

G. P. No. 277. Do. Dead in 30 minutes.

G. P. No. 278. Do. Dead in 55 minutes.

G. P. No. 279. Do. Symptoms; recovered.

G. P. No. 280. Do. Symptoms; recovered.

G. P. No. 281. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 17 days prior, $\frac{1}{250}$ c.c. antitoxic horse serum (Natl. V. 14) injected into the peritoneal cavity.]

G. P. No. 282. Do. Dead in 23 hours.

G. P. No. 283. Do. Symptoms; recovered.

G. P. No. 284. Do. Dead in 60 minutes.

G. P. No. 285. Do. Symptoms; recovered.

From the above series it will be seen that guinea pigs may be rendered as susceptible by intraperitoneal as by subcutaneous inoculations.

Finally, in order to demonstrate without doubt that neither antitoxin nor diphtheria toxine plays any part in this action, and to show that this action is a property of normal horse serum, we treated a number of guinea pigs giving them at both the first and second injection fresh normal horse serum, with the following results:

G. P. No. 101. Three c. c. normal (roan) horse serum injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 55 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (Jane).]

G. P. No. 105. One c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: As above.]

G. P. No. 104. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: As above.]

G. P. No. 102. Three c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: As above.]

G. P. No. 286. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 25 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (roan).]

G. P. No. 287. Do. Dead in 50 minutes.

G. P. No. 288. Do. Symptoms; recovered.

G. P. No. 289. Do. do do

G. P. No. 290. Do. do do

Part IV.

THE TOXIC PRINCIPLE.

At one time we made efforts to isolate the active principle in horse serum which causes the symptoms, but as soon as we realized that the toxic principle present in horse serum exerts its action in quantities so minute as to place it almost in the category of the ferments, and, further, when we concluded from our work that this toxic principle is doubtless one of those highly organized and complex proteid substances belonging to the "haptin" group, we recognized how hopeless it would be with present technique to isolate this substance. Nevertheless, we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eliminating or neutralizing this toxic principle from horse serum is at once evident.

IS THE POISON SPECIFIC?

Whether the poisonous substance in horse serum is specific may be determined in several ways. We first endeavored to determine whether guinea pigs treated with horse serum are susceptible to the serums of other aimals; and, secondly, whether guinea pigs treated with the serums of other animals are susceptible to subsequent injections of horse serum.

The following is a list of animals treated with horse serum and subsequently tested with the blood serums of other animals.

It will be noticed that in this list some of the guinea pigs were treated with donkey serum instead of horse serum, but as the donkey serum in our hands has shown itself to act the same as horse serum we have used them interchangeably.

Table showing the effect of alien serums upon guinea pigs sensitized with horse serum.

No. of guinea pig.	Kind and amount of normal serum.	Previous treatment.		Result.
		Days prior.	Inoculated subcutaneously with—	
178	Cattle, 6 c. c.	34	$\frac{1}{250}$ c. c. donkey serum	Symptoms, recovered.
149	do	41	0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. horse serum (Stearns 1351).	Do.
180	Hog, 6 c. c.	34	$\frac{1}{250}$ c. c. donkey serum	No symptoms.
138	do	41	0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. horse serum (Stearns 1351).	Symptoms, recovered.
4743	Hog, 1 c. c.	41	0.139 c. c. toxine No. 5+$\frac{1}{350}$ c. c. horse serum (Alex. 192).	Do.
179	Sheep, 6 c. c.	34	$\frac{1}{250}$ c. c. donkey serum	Do.
152	do	41	0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. horse serum (Stearns 1351).	Do.
177	Cat, 6 c. c.	34	$\frac{1}{250}$ c. c. donkey serum	Do.
124	do	41	0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. horse serum (Stearns 1351).	No symptoms.
4744	Cat, 1 c. c.	41	0.139 c. c. toxine No. 5+$\frac{1}{350}$ c. c. horse serum (Alex. 192).	Symptoms, recovered.
121	Rabbit, 6 c. c.	41	0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. horse serum (Stearns 1351).	Do.
4380	do	19	0.22 c. c. toxine No. 7+$\frac{1}{250}$ c. c. horse serum (Natl. V. 10).	Do.
4374	do	19	0.22 c. c. toxine No. 7+$\frac{1}{250}$ c. c. horse serum (Natl. V. 7).	Do.
153	Dog, 6 c. c.	41	0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. horse serum (Stearns 1351).	Do.
4379	do	19	0.22 c. c. toxine No. 7+$\frac{1}{250}$ c. c. horse serum (Natl. V. 10).	Do.
4742	Dog, 1 c. c.	41	0.139 c. c. toxine No. 7+$\frac{1}{350}$ c. c. horse serum (Alex. 192).	Do.
4375	Guinea pig, 3 c. c.	19	0.22 c. c. toxine No. 7+$\frac{1}{250}$ c. c. horse serum (Natl. V. 7).	No symptoms.

As the above series of guinea pigs previously treated with horse serum showed unmistakable susceptibility to subsequent injections of the blood serum of other animals, we are justified in saying that the action is not specific; but there is a very evident quantitative difference. It will be noticed that none of these guinea pigs died, and it was a matter of observation that, for the most part, they had characteristic but mild symptoms.

We next took up the question as to whether a guinea pig may be sensitized to horse serum with the blood serum of animals other than the horse. The following is a list of guinea pigs which had been treated by the subcutaneous inoculation of small quantities ($\frac{1}{250}$ c. c.) of blood serum from the rabbit, rat, donkey, cat, dog, hog, sheep, chicken, cattle, and man. Each pig was subsequently injected with 6 c. c. of antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity.

Table showing the results of injecting 6 c.c. of antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity of guinea pigs treated from 25 to 34 days prior with $\frac{1}{250}$ c. c. of the blood serum of other animals.

No. of guinea pig.	Previous treatment.			Result.
	Kind of serum.		Days prior.	
171	Rabbit	$\frac{1}{250}$ c.c.	25	No symptoms.
172	do	do	34	Do.
174	do	do	34	Symptoms, recovered.
167	Rat	do	25	No symptoms.
168	do	do	34	Do.
169	do	do	34	Do.
170	do	do	34	Do.
176	Donkey	do	25	Do.
191	Cat	do	25	Do.
192	do	do	34	Symptoms, recovered.
193	do	do	34	Symptoms (?), recovered.
214	Dog	do	26	No symptoms.
215	do	do	35	Do.
212	do	do	35	Symptoms, recovered.
185	Hog	do	30	No symptoms.
186	do	do	30	Symptoms, recovered.
182	Sheep	do	30	Do.
184	do	do	30	No symptoms.
196	Chicken	do	26	Symptoms, recovered.
197	do	do	26	Do.
206	Cattle	do	31	No symptoms.
207	do	do	31	Do.
210	do	do	31	Do.
201	Man	do	31	Symptoms, recovered.
202	do	do	31	No symptoms.

As the above series of guinea pigs was tested with antitoxic horse serum, we tested another series, similarly sensitized, with normal horse serum, as shown in the table on the following page, the results in all respects being similar.

Table showing the results of injecting 4 c. c. of normal horse serum (roan) into the peritoneal cavity of guinea pigs from 47 to 56 days prior with $\frac{1}{250}$ c. c. of the blood serum of other animals.

No. of guinea pig.	Previous treatment.			Result.
	Kind of serum.		Days prior.	
173	Rabbit	$\frac{1}{250}$ c. c.	55	Symptoms, recovered.
175	do	do	55	Do.
195	Cat	do	55	Do.
213	Dog	do	56	Do.
211	do	do	56	Do.
187	Hog	do	51	Do.
189	do	do	51	Do.
190	Sheep	do	47	No symptoms.
183	do	do	51	Symptoms, recovered.
181	do	do	51	Do.
199	Chicken	do	47	No symptoms.
198	do	do	47	Symptoms (?), recovered.
200	do	do	47	Symptoms, recovered.
203	Man	do	52	No symptoms.
204	do	do	52	Symptoms, recovered.
205	do	do	52	Do.

We think it is plain from the above that this action is not strictly specific for horse serum; that is, guinea pigs treated with horse serum are rendered somewhat susceptible to the subsequent injection of the serum of certain other species and that guinea pigs treated with the serum of other animals are slightly sensitive to the toxic action of horse serum. However, it is also perfectly plain from this series of experiments that the action is quantitatively specific, for it will be noticed that of the many guinea pigs sensitized with the blood serum of animals other than the horse, none died from subsequent injections of large quantities (6 c. c.) of horse serum. The same may be said of the action of these other serums upon guinea pigs previously treated with horse serum. While most of these serums produced symptoms, none of the guinea pigs died.

In other words, there is plainly a distinct quantitative difference, both in the results of the power of horse serum to render guinea pigs susceptible to other serums and in its toxic action upon guinea pigs sensitized by other serums.

The action of horse serum upon other animals than the guinea pig is treated of in another chapter (p. 55).

ARE OTHER BLOOD SERUMS EQUALLY TOXIC?

We now know that guinea pigs previously treated with small injections of horse serum become very susceptible to subsequent injections of horse serum, but only slightly susceptible to the blood serum of other animals. It becomes important to determine whether guinea pigs treated with the serum of other animals (dog, hog, rat, cattle, sheep, cat) become as highly sensitized to a second injection of the homologous serum.

The following series of guinea pigs were all sensitized with 1/250 c. c. of the blood of various animals. Subsequently they were injected with horse serum to which they either did not respond or, after characteristic symptoms, recovered. These guinea pigs were finally injected with large quantities of the same kind of serum as that used at the first injection.

Dog serum.

G. P. No. 214. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 26 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *dog* serum.]

5 hrs. later 6 c. c. *dog* serum injected into the peritoneal cavity. Symptoms.

G. P. No. 215. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 35 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *dog* serum.]

22 days later 6 c. c. *dog* serum injected into peritoneal cavity. Severe symptoms.

G. P. No. 212. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Mild symptoms.

[Previous treatment: 35 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *dog* serum.]

22 days later 6 c. c. *dog* serum injected into peritoneal cavity. Severe symptoms.

G. P. No. 211. Four c. c. normal *horse* serum (roan) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 56 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *dog* serum.]

1 day later 6 c. c. *dog* serum injected into the peritoneal cavity. Dead in 35 minutes

Hog serum.

G. P. No. 186. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Severe symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *hog* serum.]

22 days later 6 c. c. *hog* serum injected into the peritoneal cavity. Severe symptoms.

G. P. No. 185. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *hog* serum.]

22 days later 5 c. c. *hog* serum injected into the peritoneal cavity. No symptoms.

G. P. No. 187. Four c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 51 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *hog* serum.]

1 day later 6 c. c. *hog* serum injected into the peritoneal cavity. Symptoms.

G. P. No. 189. Same injection and previous treatment. No symptoms.

1 day later 6 c. c. *hog* serum injected into the peritoneal cavity. Symptoms.

Cattle serum.

G. P. No. 206. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 31 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *cattle* serum.]

22 days later 6 c. c. *cattle* serum injected into the peritoneal cavity. Dead in 120 minutes.

G. P. No. 210. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 31 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *cattle* serum.]

22 days later 6 c. c. *cattle* serum injected into the peritoneal cavity. Dead in 45 minutes.

G. P. No. 207. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 31 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *cattle* serum.]

22 days later 6 c. c. *cattle* serum injected into the peritoneal cavity. Dead in 90 minutes.

Sheep serum.

G. P. No. 184. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *sheep* serum.]

22 days later 6 c. c. *sheep* serum injected into the peritoneal cavity. Severe symptoms; dead in 23 hours.

G. P. No. 182. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Severe symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *sheep* serum.]

22 days later 6 c. c. *sheep* serum injected into the peritoneal cavity. Severe symptoms.

G. P. No. 190. Four c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 47 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *sheep* serum.]

1 day later 6 c. c. *sheep* serum injected into the peritoneal cavity. Severe symptoms.

G. P. No. 183. Four c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 51 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *sheep* serum.]

1 day later 6 c. c. *sheep* serum injected into the peritoneal cavity. Dead in 18 hours.

G. P. No. 181. Same injection and previous treatment. Mild symptoms.

1 day later 6 c. c. *sheep* serum injected into the peritoneal cavity. Severe symptoms. Dead in 21 hours.

Cat serum.

G. P. No. 192. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Severe symptoms.

[Previous treatment: 34 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *cat* serum.]

22 days later 6 c. c. *cat* serum injected into the peritoneal cavity. Severe symptoms.

G P. No. 195. Four c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 55 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. *cat* serum.]

1 day later 6 c. c. *cat* serum injected into the peritoneal cavity. Dead in 35 minutes.

Rat serum.

G. P. No. 168. Six c. c. antitoxic *horse* serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 34 days prior inoculated subcutaneously with $\frac{1}{250}$ c. c. *rat* serum.]

2 hours later 2 c. c. *rat* serum injected into the peritoneal cavity. Severe symptoms. Dead in 3 hours.

We infer from the above series that guinea pigs treated with the serums of various animals and subsequently injected are much more susceptible to homologous serums than to heterologous serums. We have here another indication that this toxic action of blood serum is quantitatively specific.

We expect to take up the work with the serums of other animals more in detail, and the results will be reported at another time.

THE RELATION OF HEMOLYSIS.

So much work has recently been done indicating that the toxic property of blood serum is closely allied with its hemolytic action that we made some experiments in order to determine what connection exists between the hemolytic and toxic action of horse serum upon guinea pigs. As a result of these studies we believe that we have shown very clearly that blood serum may contain an acute poison entirely independent of any hemolytic action.

Landois [a] in his work on transfusion showed long ago (1875) that the blood serum of many mammals dissolves the red blood cells of other mammals. Landois specifically demonstrated that the red blood corpuscles of rabbits, when mixed with alien blood serum, are readily dissolved, while, on the other hand, the red blood corpuscles of cats and dogs are much more resistant. If large quantities of red blood corpuscles are dissolved, there follows an excretion of hemoglobin through the kidneys, intestines, and also into the serous cavities. If the serum is injected into the blood stream of a rabbit, some of the erythrocytes of the rabbit are dissolved in vivo, as may readily be confirmed microscopically; but before the corpuscles are dissolved there is an agglutination of the blood corpuscles into clumps, by means of which large capillary areas may become plugged. The same process is seen by the further extraction of hemoglobin from the mass of the stroma of the corpuscles which fills the capillaries. The dissolved hemoglobin now causes further blocking of the vessels by means of clots, which are formed through the action of the hemoglobin upon the leucocytes, freeing the fibrin ferment. From this somewhat complicated process there may result serious symptoms, as dyspnea, convulsions, etc., in proportion to the amount of blood injected. If the amount of blood is large, death may follow, as Landois has shown, with phenomena of asphyxiation. (Uhlenhuth: Zur Kenntniss der giftigen Eigenschaften des Blutserums. Zeit. f. Hyg., vol. 26, 1897, p. 384.)

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[a] Landois: Lehrbuch der Physiologie des Menschen, 1896. Die Transfusion des Blutes. Leipzig, 1875.

Hermann Pfeiffer,[a] confirming the work of Uhlenhuth upon the necrotic action of blood serums when injected subcutaneously into guinea pigs, believes that the toxic substance producing the necrosis is the same as the hemolytic agent found in the blood serum.

In recent years hemolysis has been studied more particularly by Ehrlich, Morgenroth, Bordet, Metchnikoff, and many others. Much of the work done appears to corroborate the early observations of Landois, who believed that the toxic action of blood serum when injected intravenously depends upon a "globulicidal" action upon the red blood cells and the plugging of the capillaries. On the other hand, we have the views of other authors based in part upon experimental data that the toxic action of blood serum is dependent upon albuminous substances independently of the lytic substance present in the serum.

Creite[b] in 1869 was aware of Landois's view concerning hemolysis, but separated the toxic power from the blood serum by means of coagulation (heat).

Also, Weiss[c] in 1896 showed by separating albuminous substances from the blood serum by means of heating or absolute alcohol that the serum has no longer the toxic power and concluded, therefore, that the toxic action is brought about by the albumins.

Albu[d] separated the albuminous substances from blood serum by precipitation with ammonium sulphate, which he dialyzed out, as it is itself poisonous, and came to the same conclusion as Creite and Weiss.

Rummo[e] believed that the toxic substance is a tox-albumin which, according to his views, is formed in the living cells of the respective animals.

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[a] Hermann Pfeiffer: Über die nekrotisirende Wirkung normaler Seren. Zeit. f. Hyg., 1905, Band 150, p. 183.

[b] Creite: Versuche über die Wirkung des Serumweisses nach Injection in das Blut. Zeit. f. rationelle Med. Bd. 36, S. 90.

[c] Weiss: Ueber die Wirkungen von Blutserum-Injectionen in's Blut. Pflüger's Arch., Bd. 65, 1896.

[d] Albu: Untersuchungen über die Toxicitat normaler u. pathol. Serumflüssigkeit. Virchow's Arch., Bd. 149, 3.

[e] Rummo: Ueber die Giftigkeit des Blutserums bei Menschen und Thieren im normalen Zustande und bei Infectionskrankheiten. Wiener med. Woch., 1891, nr. 19–20.

Mairet and Bosc [a] were able to distinguish in blood serum a substance capable of producing coagulation and another substance capable of producing poisonous symptoms. They believed both of these substances to be albumins. They were able to eliminate the coagulation-producing substance by heating from 52° to 53° C. or by the addition of sodium chloride and sodium sulphate.

Pearce [b] believes that it appears to be definitely established that red blood corpuscle thrombi occur in the experimental lesions caused by sera possessing hemagglutinative power. He demonstrated that the capillaries of certain areas in the liver are entirely occluded by fused red cells, and he also showed the inability of the products of hemolysis to produce such action and necrotic areas in the liver.

L. Camus and E. Gley [c] first endeavored to separate the hemolytic action from the toxic action of blood serum by means of heating. They found that heat destroys the hemolytic action; but while it diminishes it does not entirely destroy its general toxic action. This work was done with eel serum. In their previous work the two actions seemed about parallel. For instance, eel serum is very hemolytic for rabbits and guinea pigs and equally poisonous for these animals. On the other hand, the red blood cells of the pigeon and those of the porcupine are very resistant to eel serum, and these animals also resist doses which are fatal for the rabbit and the guinea pig. However, these authors finally found an animal, the marmot, for which eel serum had slight hemolytic action but was extremely toxic, and they believed that these researches upon the marmot have permitted them to separate the toxic action of eel serum from its hemolytic action better than did their previous work by means of heating.

We found horse serum to have practically no hemolytic action upon the corpuscles of the guinea pig. This applies with equal force to antitoxic horse serum as to normal horse serum, as may be seen from the following table:

Hemolysis.

G. P. No. 2. Normal pig. Serum obtained by coagulation.
G. P. No. 1. Normal pig. Copuscles obtained by centrifugation, etc.

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[a] Mairet and Bosc: Recherches sur les causes de la toxicité du serum du sang. Compt. rend., t. 119, 4, p. 292. Toxicité du serum du sang de l'homme sain., Compt. rend., 1897. Des effects de la chaleur sur la toxicité du sérum. Compt. rend. July 7, 1894.

[b] Pearce, Richard M.: A further study of the experimental production of liver necrosis by the injection of hemagglutinative sera. J. M. Research, v. 14, Apr., 1906, p. 541. Pearce, R. M., and Winne, C. K., jr.: Concerning hemagglutins of bacterial origin and their relation to hyalin thrombi and liver necroses. Am. J. M. Sci., v. 128, 1904, p. 669.

[c] Camus, L., and Gley, E.: Comparison between the hemolytic and the toxic action of eel's serum in the marmot (*Arctomys marmota*). Arch. internat. de pharmacodyn. et de therapie, tome 15, fasc. 1 and 2, 1905.

G. P. No. 4517. Serum obtained by coagulation.

[Previous treatment: 35 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. 4510. Corpuscles obtained by centrifugation, etc.

[Previous treatment: 35 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

1 c. c. of—	1 c.c., 5 per cent corpuscles, normal guinea pig No. 1 (washed).			1 c. c., 5 per cent corpuscles, treated guinea pig No. 4510 (washed).		
		Do. +1 gtt. serum normal guinea pig No. 2.	Do. +1 gtt. serum guinea pig No. 4517.		Do. +1 gtt. serum normal guinea pig No. 2.	Do. +1 gtt. serum guinea pig No. 4517.
Normal horse serum (Sam.)	0	0	0	0	0	0
Normal donkey serum	Faintest trace.	0	0	0	0	0
Normal horse serum (Sp.)	0	Faintest trace.	Faintest trace.	0	0	Faintest trace.
Antitoxic horse serum (Natl. VIII. 17)	0	0	0	0	0	0
Antitoxic horse serum (Natl. IX. 19)	0	0	0	0	0	0
Antitoxic horse serum (Natl. V. 14)	0	0	0	0	0	0
Antitoxic horse serum (Alex. A205, Gibson's method).	Trace.	Trace.	Trace.	Trace.	Trace.	Trace.
Salt solution (control)	0	Trace.	0	0	0	0

First the corpuscles were placed in the test tubes, then the various horse serums, and finally the drop of guinea-pig serum.

The corpuscles were obtained by whipping the blood and washing in the usual way four times and suspended so as to make the so-called 5 per cent suspension. After the corpuscles and the serum were mixed they were well shaken and placed in the incubator, again shaken at the end of one hour, again at the end of two hours, and at the end of three hours taken out of the incubator and placed in the ice chest. The readings were taken the next morning.

This work was repeated several times until we were satisfied that neither the normal horse serum nor the antitoxic horse serum had practically any power of dissolving the hemoglobin from the guinea-pig corpuscles. There can therefore be no doubt that there is a toxic substance in the blood serum of horses entirely independent of any hemolytic action. As a further corroboration we may state that autopsies upon guinea pigs which have died from the results of the injections of horse serum showed none of the usual effects, indicating that hemolysis took place within the body.

Further, horse serum heated to 60° for six hours is as toxic as unheated serum.

As a further study of the relation of hemolysis to the toxic action and also of the relation of the toxic principle to the haptin group we made the following experiments:

G. P. No. 249. Four subcutaneous injections of 1.5 c c. each on successive days, using normal roan horse corpuscles washed three times. The blood serum of this guinea pig developed a high degree of hemolytic power against horse corpuscles. The blood serum of this guinea pig was mixed with equal quantities of normal horse serum and the mixture injected into the following three guinea pigs:

G. P. No. 4993. Two c. c. of the above mixture injected into the peritoneal cavity. Dead in 40 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{550}$ c. c. antitoxic horse serum (Alex. A206).]

G. P. No. 4988. Same injection. Dead in 30 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{150}$ c. c. antitoxic horse.serum (Alex. A202).]

G. P. No. 4992. Same injection. Dead in 35 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneuosly with 0.22 c. c. toxine No. $7+\frac{1}{500}$ c. c. antitoxic horse serum (Alex. A208).]

The above experiment was repeated as follows:

G. P. No. 248. Four subcutaneous injections of 1.5 c. c. each on successive days were made, using roan corpuscles washed three times. The blood serum of this guinea pig developed a high degree of hemolytic power against horse corpuscles. The serum was mixed with equal quantities of normal horse serum and the mixture injected into the following guinea pigs:

G. P. No. 4913. Four c. c. of the above mixture injected into the peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 41 days prior, inoculated subcutaneously with 0.139 c. c. of toxine No. $5+\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 12D).]

G. P. No. 4914. Two c. c. same serum injected into the peritoneal cavity. Dead in 20 minutes.

[Previous treatment: Same as G. P: No. 4913.]

THE RELATION OF PRECIPITIN TO THE TOXIC ACTION.

The precipitins are so specific and so closely related to the cytotoxins that it natually occurred to us to determine what relation there may exist between the toxic power of the blood serum of the horse and of the guinea pig, and the power of these two serums to precipitate each other.

If we inject horse serum several times into a guinea pig we would expect to find that the blood serum of a guinea pig so treated would have a power of precipitating horse serum. This we found to be the case; but we could not find that the injection of a single small dose of horse serum into the guinea pig developed this power in the guinea pig's blood serum. We might, however, conjecture that the union of the two serums under the conditions named causes a precipitation which is not visible, or causes a chemical union between two highly organized albuminous substances having toxic properties. In order to test this subject more thoroughly we first made some experiments to determine the precipitating action of normal and antitoxic horse serum upon normal guinea-pig serum, as follows:

One c. c. of horse serum in dilution of 1:21 was placed in a small test tube. The 0.1 c. c. of normal guinea-pig serum was added to each tube. These were thoroughly shaken, placed in the incubator, and the results read after twenty-four hours.

Normal G. P. No. 1. Killed the same day with chloroform, blood defibrinated, and the serum separated in the centrifuge.

Normal G. P. No. 2. Killed by chloroform 3 days previously and the serum allowed to separate by coagulation.

(Two tests made of each serum.)

1 c. c. horse serum (1:21).	0.1 c. c. serum normal guinea pig 1.	0.1 c. c. serum normal guinea pig 2.
Normal horse serum (Sam)	Nil. Nil.	Nil. Nil.
Normal horse serum (Jane)	Nil. Nil.	Nil. Nil.
Antitoxic horse serum (Natl. VIII, 17)	Nil. Nil.	Nil. Nil.
Antitoxic horse serum (Hubbert op. 1)	Nil. Slight precipitation(?).	Nil. Nil.
Antitoxic horse serum (Natl. IX, 17)	Nil. Nil.	Nil. Nil.
Antitoxic horse serum (Stearns 1110)	Nil. Nil.	Nil. Nil.

From this and other similar experiments we are justified in concluding that neither normal horse serum nor antitoxic horse serum has any power to precipitate normal guinea-pig serum.

We next made some tests to determine whether a guinea pig previously treated with one small injection of horse serum developed precipitins.

One c. c. of horse serum both normal and antitoxic in dilution of 1:21 was placed in small tubes, as before, and 0.1 c. c. of a sensitized guinea-pig serum added to each tube, as shown in the table. They were shaken and placed in the incubator and results read after twenty-four hours.

G. P. No. 4617. [Previous treatment: 35 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (standard B27).]

G. P. No. 4510. [Previous treatment: same.]

1 c.c. horse serum (1:21).	0.1 c.c. normal guinea-pig serum.		0.1 c. c. treated guinea-pig serum.	
	Centrifugation.	Coagulation.	Guinea pig 4517.	Guinea pig 4510.
Normal horse serum (Sam)	0	0	0	0
Normal horse serum (Jane)	0	0	0	0
Normal horse serum (S. P.)	0	0	0	0
Normal donkey serum	0	0	0	0
Antitoxic horse serum (Natl. VIII, 17)	0	0	0	0
Antitoxic horse serum (Natl. V, 14)	0	0	0	0
Antitoxic horse serum (Natl. IX, 17)	+(?)	0	0	0
Antitoxic horse serum (Stearns 1110)	0	0	0	0
Antitoxic horse serum (Stearns 1429)	0	0	0	0

It would therefore appear that guinea pigs which have previously been inoculated with one injection of a very small amount of horse serum sufficient to render them highly susceptible to a second injection of horse serum do not contain sufficient precipitin in their blood serum to cause a visible precipitation when added to horse serum.

The following studies show still more clearly that the specific precipitins have nothing to do with this toxic reaction.

We prepared guinea pigs by injecting them many times at short intervals with horse serum, so as to develop a high degree of precipitin in their blood serum against horse serum. The blood serum of guinea pigs so prepared was then added to horse serum in the proportions of about one of the former to two of the latter and allowed to stand until the precipitating action was complete. This guinea-pig serum was so active that very large quantities of the precipitum settled to the bottom of the test tubes. The mixture was now filtered, so as to separate the precipitum entirely, and the filtrate injected into sensitized guinea pigs and found to be quite as toxic as untreated horse serum.

G. P. No. 107. Seventeen subcutaneous injections of 1 c. c. each normal horse serum (roan) and one peritoneal injection of 6 c. c. antitoxic horse serum (Natl. IX, 19) were given between February 8 and March 11.

Six c. c. normal horse serum (roan) were mixed with 3 c. c. of the serum of this guinea pig and allowed to stand about 3 hours at room temperature. The large amount of precipitum which developed was filtered off and the filtrate injected into the following two guinea pigs:

G. P. No. 4996. Two c. c. of the above filtrate injected into peritoneal cavity. Dead in 20 minutes.

[Previous treatment: 27 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{700}$ c. c. antitoxic horse serum (Wellcome 474A).]

G. P. No. 5000. Five c. c. of the above filtrate injected into the peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{700}$ c. c. antitoxic horse serum (Wellcome 477B).]

These experiments were repeated under varying conditions, especially allowing the two serums to remain in contact a longer time before filtering, and similar results obtained.

The results of this part of our work are strengthened by the conclusions of Pirquet and Schick, who found that the antibodies, whatever they may be, producing symptoms of the serum disease, are not identical with the precipitins. They found, for instance, that the symptoms of the serum disease appear within eight to thirteen days following the first injection of horse serum into man, whereas it requires about three weeks for precipitins to appear in the blood in children after the injection of horse serum.

The formation of precipitins does not take place as readily in man following the injection of horse serum as it does in rabbits. In fact, Pirquet and Schick found that sometimes even after the injection of 200 c. c. there was no production of precipitins, and, further, that the length of time that the precipitin reaction remains in the blood of man following the injection is variable. It sometimes disappears in the fourth week, and sometimes is still markedly present in the ninth week.

Finally, we know that the precipitins are strictly specific, whereas the toxic action is only quantitatively specific.

EFFECT OF HEAT UPON THE TOXICITY OF HORSE SERUM.

One of the first questions which required solution was whether the toxic substance in blood serum is thermolabile. That it is able to withstand a comparatively high degree of heat for a long time, viz. 60° C. for six hours, is evident from the following experiments:

G. P. No. 4349. Six c. c. antitoxic horse serum (Natl. VIII. 17) heated to 60° C. for 30 minutes, then injected into the peritoneal cavity. Dead in 30 minutes.

G. P. No. 60. One c. c. same serum and injection as g. p. 4349. Dead in 10 minutes.

[Previous treatment: With toxine-antitoxin mixture.]

G. P. No. 61. Do. Dead in 10 minutes.

G. P. No. 62. Do. Symptoms; recovered.

G. P. No. 4897. Injected with 5 c. c. of an extract of normal horse serum (roan) heated to 60° C. for 6 hours. This degree of heat coagulated the serum. About 1 c. c. of the coagulum was added to 9 c. c. of salt solution, thoroughly shaken up, filtered, and the filtrate injected into the peritoneal cavity. Symptoms; death in about 3 hours.

[Previous treatment: 41 days prior, injected subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{360}$ c. c. horse serum (Alex. No. 192).]

G. P. No. 4508. Six c. c. of an extract of antitoxic horse serum (Natl. IX. 19) heated to 100° C. for 15 minutes, injected into the peritoneal cavity. The coagulum was ground up with a few c. c. of salt solution and strained through gauze before being injected. No symptoms.

One and a half hours later about 8 c. c. of the coagulum was ground up and the entire mass, not strained, injected into the peritoneal cavity of the same guinea pig. No symptoms.

G. P. No. 5048. Six c. c. of the coagulum of normal horse serum (roan) heated to 100° C. for 15 minutes and ground up with salt solution (0.85) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 20 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{500}$ c. c. antitoxic horse serum (Alex. A204).]

From the above it is evident that while a temperature of 60° C. continued for six hours has practically no influence upon the toxic substance in horse serum, the toxicity is entirely destroyed by a temperature of 100° C. for fifteen minutes.

THE TOXIC PRINCIPLE IN HORSE SERUM IS FILTERABLE THROUGH PORCELAIN.

The following experiment is sufficient to demonstrate that the close-grained pores of a Pasteur-Chamberland filter B do not retain the toxic principle of horse serum:

G. P. No. 4521. Six c. c. of antitoxic horse serum (Natl. VIII. 17) filtered through a Pasteur-Chamberland filter B and injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 32 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

DRYING DOES NOT INJURE THE TOXIC PRINCIPLE.

We have on hand a large quantity of dried antitoxic horse serum which has been prepared and preserved under special conditions for the preparation of the standard antitoxic unit. This serum was carefully dried under bacteriological precautions at a temperature below 37° C., as described in Bulletin No. 21.[a]

That such dried and redissolved serum still retains its toxic property is evident from the following experiment:

G. P. No. 4376. Six c. c. antitoxic horse serum (B 1+12) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 19 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{250}$ c. c. horse serum (Natl. V. 7).]

THE TOXIC PRINCIPLE CAN NOT BE SEPARATED BY PRECIPITATION AND DIALYSIS.

We made several experiments to determine whether precipitation by ammonium sulphate and subsequent dialysis to remove the sulphate and other inorganic salts would also remove the toxic principle. For this purpose we used antitoxic horse serum prepared and dialysed in accordance with Gibson's method.[b] It is plain from the following experiments that precipitation and dialysis do not remove the toxic principle:

G. P. No. 4104. Six c. c. antitoxic horse serum (NYBH A) injected into the peritoneal cavity. Dead in 5 minutes.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{250}$ c. c. horse serum (Alex. 190).]

G. P. No. 4506. Five c. c. antitoxic horse serum (Alex. A 203/NYBH) injected into the peritoneal cavity. No symptoms.[c]

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4513. Five c. c. antitoxic horse serum (Alex. A 203/NYBH) injected into the peritoneal cavity. Dead in 39 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

EFFECT OF VARIOUS CHEMICAL SUBSTANCES UPON THE TOXIC PRINCIPLE OF HORSE SERUM.

Various substances of widely different chemical nature were added to horse serum, in order to see whether any of them had the power of precipitating, oxidizing, or neutralizing the toxic property of the serum. The following substances were used: Succinic acid peroxide (alphozone), butyric acid, hydrogen dioxide, permanganate of potash, alcohol, ammonium sulphate, and citrate of soda.

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[a] Rosenau, M. J.: The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum); official standard prepared under the act approved July 1, 1902. Bulletin 21, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service. Washington, Government Printing Office, April, 1905.

[b] Gibson, R. B.: On the practical concentration of diphtheria antitoxin. Am. med., vol. 10, 1905, p. 915.

[c] Explanation of occasional irregularities, p. 63.

Butyric acid.

G. P. No. 4368. Ten c. c. of a $\frac{N}{10}$ butyric acid solution were added to 25 c. c. antitoxic horse serum (Natl. IX. 17); the mixture was kept at 15° C. for 40 hours, at the end of which time the fine precipitate which developed was filtered off and 3 c. c. injected into the peritoneal cavity. Dead in 2 hours 10 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{330}$ c. c. horse serum (Cutter 1461).]

Hydrogen dioxide.

G. P. No. 4371. Five c. c. of hydrogen dioxide (Oakland) were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours, at the end of which time a very little fine precipitate had formed, but the mixture effervesced in filtering; 3 c. c. of the filtrate was injected into the peritoneal cavity. Symptoms (?); recovery.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{270}$ c. c. horse serum (Cutter 1504).]

G. P. No. 4917. Five c. c. hydrogen dioxide (McKesson & Robbins) were added to 25 c. c. antitoxic horse serum (Natl. IX. 17); the mixture was kept at 15° C. for 40 hours, at the end of which time the fine precipitate which developed was filtered off, and 6 c. c. injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 41 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{180}$ c. c. antitoxic horse serum (Stearns 16H).]

Permanganate of potash.

G. P. No. 4370. Three c. c. of a 1 per cent solution of permanganate of potash were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours, and as no precipitate had formed in that time it was not filtered; 3 c. c. of the mixture inoculated into the peritoneal cavity. Dead in 45 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{430}$ c. c. horse serum (Cutter 1461).]

Succinic peroxide acid (alphozone).

G. P. No. 4372. Five c. c. of a 1 per cent solution of alphozone were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours; the precipitate which formed was filtered off and 3 c. c. of the filtrate injected into the peritoneal cavity. No symptoms.[a]

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. horse serum (Cutter 1504).]

G. P. No. 5A. Ten c. c. of the same filtrate injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 30 days prior, inoculated with 0.22 c. c. toxine No. 7+1 unit of horse serum (Standard B27).]

As we found that 2 c. c. of a 1 per cent solution of succinic peroxide acid (alphozone) injected into the peritoneal cavity of a normal guinea pig produced transient irritating effects these experiments were repeated, using smaller quantities of the acid, as follows:

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[a] Explanation of occasional irregularities, p. 63.

G. P. No. 4519. One c. c. of a 1 per cent solution of alphozone was added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15 C. for 40° hours; the precipitate which formed was filtered off and 6 c. c. of the filtrate injected into the peritoneal cavity. Dead in 24 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4518. Five c. c. added as above and 6 c. c. of the filtrate injected into the peritoneal cavity. Dead in 25 minutes.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

Alcohol.

G. P. No. 4373. Five c. c. of 95 per cent alcohol were added to 25 c. c. antitoxic horse serum (Natl. IX. 19); the mixture was kept at 15° C. for 40 hours; the precipitate which formed was filtered off and 3 c. c. of the filtrate injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{370}$ c. c. horse serum (Cutter 1504).]

G. P. No. 5B. Seven and a half c. c. added as above and 3 c. c. of the filtrate injected into the peritoneal cavity. No symptoms.[a]

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 156. Seven and a half c. c. added as above and 6 c. c. of the filtrate injected into the peritoneal cavity. Dead in 90 minutes.

[Previous treatment: 60 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 4363. Ten c. c. added as above and 3 c. c. of the filtrate injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{250}$ c. c. horse serum (P D & Co .07173–G).]

G. P. No. 4C. Twenty-five c. c. added as above. This caused practically solid coagulation on the serum. The coagulent was vigorously shaken up with salt solution (0.85) and filtered by the aid of a vacuum, and 6 c. c. of the opalescent filtrate injected into the peritoneal cavity. Dead in 4 hours 10 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

Ammonium sulphate.

We know from the work done with serum precipitated with ammonium sulphate and subsequently dialysed that the ammonium sulphate has no action upon the toxic principle found in horse serum. (See guinea pigs Nos. 4104, 4106, and 4513, p. 39.)

Citrate of soda.

We know that citrate of soda has no injurious action upon the toxic principle found in horse serum because some of the serum we used was prepared by adding 1 per cent solution of citrate of soda to the whole blood as it was drawn from the horse in order to prevent clotting and the clear serum subsequently pipetted off. The citrate of soda was afterwards removed by dialysis (Gibson's method) and the plasma proved just as toxic to treated guinea pigs as other horse serum. We also have the following experimental data upon this point:

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[a] For explanation of occasional irregularities, see p. 63.

G. P. No. 4925. Six c. c. normal horse serum (roan) containing 1 per cent citrate soda injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{500}$ c. c. antitoxic horse serum (Cutter 1465).].

G. P. No. 4918. Injected as above. Dead in 5 minutes.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{230}$ c.c. antitoxic horse serum (Stearns 16 H).]

G. P. (control). The above guinea pigs were controlled by injecting 6 c. c. of the same serum containing 1 per cent citrate of soda into the peritoneal cavity of a normal pig. The only effects of this inoculation were symptoms of irritation which soon passed away.

INFLUENCE OF ANTISEPTICS, ETC.

As much of this work was done with horse serum preserved by means of chloroform and trikresol (0.4 per cent) we made a control experiment, using horse serum as fresh as possible without the addition of any substance. The following experiment eliminates these factors as having any influence upon the toxicity of the serum:

G. P. No. 125. Three c. c. normal horse serum (roan) freshly drawn, centrifugized and injected into the peritoneal cavity as soon as the serum separated. Dead in 45 minutes.

[Previous treatment: 49 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 1351).]

EFFECT OF OLD HORSE SERUM UPON SUSCEPTIBLE GUINEA PIGS.

At one time in our work it apperaed to us that the toxic properties of horse serum gradually diminished in toxicity with the age of the serum. It was, however, soon proved by the following experiments that very old serums may be quite as poisonous as those freshly drawn.

G. P. No. 4496. Four c.c. antitoxic horse serum (Natl. V. 2) injected into the peritoneal cavity. The serum was at least *one and a half years old* when injected. Dead in 30 minutes.

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit of horse serum (Standard B27).]

G. P. No. 4921. Six c. c. antitoxic horse serum *over 3 years old* (P. D. & Co .0495 prepared Dec. 15, 1902) inoculated into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{300}$ c. c. antitoxic horse serum (Cutter 1465).]

G. P. No. 4922. Same injection. No symptoms.[a]

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{400}$ c. c. antitoxic horse serum (Cutter 1465).]

G. P. No. 4899. Same injection. Dead in 6 minutes.

[Previous treatment: 48 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{350}$ c.c. antitoxic horse serum (Alex. 192).]

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[a] Explanation of occasional irregularities, p. 63.

The following guinea pigs were inoculated with horse serum eight and one-half years old:

G. P. No. 258. Six c. c. antiamarillic serum injected into the peritoneal cavity. Dead in 40 min.

[Previous treatment: 24 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (roan) heated to 60° C. for two hours.]

G. P. No. 256. Same injection. Symptoms, recovered.

[Previous treatment: 20 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (roan) heated to 60° C. for two hours.]

From the above experiments we see that blood serum over eight and one-half years old is as poisonous to sensitized pigs as fresh serum.

EFFECT OF X-RAYS UPON THE TOXIC PRINCIPLE.

Normal horse serum (roan) was exposed to the action of X rays, 40 amperes, 4 inches from the tube for forty minutes, then injected into a susceptible guinea pig. It was found as poisonous as horse serum not so treated.

G. P. No. 6A. Six c.c. above serum injected into peritoneal cavity. Dead in 10 minutes.

[Previous treatment: 33 days prior, inoculated subcutaneously with 0.293 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B28).]

Part V.

THE INFLUENCE OF TIME.

TIME NECESSARY TO RENDER A GUINEA PIG SUSCEPTIBLE.

We know from our preliminary work that some time is necessary after the first injection of horse serum before a guinea pig is rendered susceptible to the toxic action of a second injection. The following series demonstrates that this time is about ten days:

G. P. No. 4464. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.
[Previous treatment: *5 days* prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4485. Six c. c. same serum and injection as g. p. 4484. No symptoms.
[Previous treatment: *6 days* prior, as g. p. 4484.]

G. P. No. 4486. Six c. c. same serum and injection as g. p. 4484. No symptoms.
[Previous treatment: *7 days* prior, as g. p. 4484.]

G. P. No. 4533. Six c. c. same serum and injection as g. p. 4484. No symptoms.
[Previous treatment: *8 days* prior, as g. p. 4484.]

G. P. No. 4530. Six c. c. same serum and injection as g. p. 4484. Symptoms; recovered.
[Previous treatment: *10 days* prior, as g. p. 4484.]

G. P. No. 4532. Six c. c. same serum and injection as g. p. 4484. Symptoms; recovered.
[Previous treatment: *11 days* prior, as g. p. 4484.]

G. P. No. 4488. Six c. c. same serum and injection as g. p. 4484. Dead in 10 minutes.
[Previous treatment: *12 days* prior, as g. p. 4484.]

G. P. No. 4525. Six c. c. same serum and injection as g. p. 4484. Dead in 45 minutes.
[Previous treatment: *12 days* prior, as g. p. 4484.]

G. P. No. 4487. Six c. c. same serum and injection as g. p. 4484. Dead in 60 minutes.
[Previous treatment: *13 days* prior, as g. p. 4484.[

G. P. No. 4531. Six c. c. same serum and injection as g. p. 4484. Dead in 45 minutes.
[Previous treatment: *13 days* prior, as g. p. 4484.]

G. P. No. 4476. Six c. c. same serum and injection as g. p. 4484. Dead in 10 minutes.
[Previous treatment: *14 days* prior, inoculated subcutaneously with 0.22 c. c. toxine No 7+$\frac{1}{200}$ c. c. antitoxic serum (Natl. IX. 18).]

G. P. No. 4490. Six c. c. same serum and injection as g. p. 4484. Dead in 46 minutes.
[Previous treatment: *14 days* prior, as g. p. 4484.]

G. P. No. 4529. Six c. c. same serum and injection as g. p. 4484. Dead in 47 minutes.
[Previous treatment: *15 days* prior, as g. p. 4484.]

THE GUINEA PIG REMAINS SUSCEPTIBLE A VERY LONG TIME.

That the guinea pig remains susceptible to the toxic action of horse serum a very long time is indicated in the following experiments in which 160 days elapsed between the first treatment and the second injection. How much longer this action may continue will be ascertained and reported upon at a subsequent time.

G. P. No. 4507. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Dead in 20 minutes.

[Previous treatment: 60 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit horse serum (Standard B27).]

G. P. No. 4A. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 22 minutes.

[Previous treatment: 84 days prior, inoculated subcutaneously with 0.293 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 3827. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 33 minutes.

[Previous treatment: 160 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{350}$ c. c. horse serum (Jordan No. 201).]

Part VI.

DOSAGE AS A FACTOR.

MINIMAL AMOUNT OF HORSE SERUM NECESSARY TO RENDER A GUINEA PIG SUSCEPTIBLE.

A large part of our work was done upon guinea pigs which had been used to measure the strength of antitoxic serums. As the strength of diphtheria antitoxin runs between 250 and 1,000 units to the c. c. most of our guinea pigs were therefore rendered susceptible by doses of horse serum varying from $\frac{1}{250}$ to $\frac{1}{1000}$ c. c. It now becomes interesting to know how small a quantity of horse serum injected into a healthy guinea pig would render it susceptible to the toxic action of horse serum.

G. P. No. 217. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with $\frac{1}{100}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 219. Do.

G. P. No. 216. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with $\frac{1}{100}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 222. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with $\frac{1}{1000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 223. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 130 minutes.

[Previous treatment: 45 days prior, inoculated subcutaneously with $\frac{1}{1000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 220. Do. Dead in 68 minutes.

G. P. No. 225. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 24 days prior, inoculated subcutaneously with $\frac{1}{10000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 224. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with $\frac{1}{10000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 227. Do.

G. P. No. 229. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.

Previous treatment: 24 days prior, inoculated subcutaneously with $\frac{1}{100000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 230. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with $\frac{1}{100000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 228. Do.

G. P. No. 232. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 24 days prior, inoculated subcutaneously with $\frac{1}{1000000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 235. Do.

G. P. No. 234. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with $\frac{1}{1000000}$ c. c. antitoxic horse serum (Natl. IX. 19).]

G. P. No. 233. Do. Symptoms; recovered.

It is plain from the above series that guinea pigs may be sensitized with very small amounts of horse serum. Guinea pig No. 233 received only $\frac{1}{1000000}$ of a c. c. at the first injection and this quantity was sufficient to render it susceptible to a subsequent injection of horse serum.

MINIMAL AMOUNT OF HORSE SERUM NECESSARY TO POISON A SUSCEPTIBLE GUINEA PIG.

From the following series of guinea pigs it will be seen that the toxic principle must be present in exceedingly small quantities of horse serum.

G. P. No. 4348. Four c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 30 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{440}$ antitoxic horse serum (PD 08021).]

G. P. No. 4099. Three c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{110}$ c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 3951. Three c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 57 minutes.

[Previous treatment: 51 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{310}$ c. c. antitoxic horse serum (Hubbert 1).]

G. P. No. 4364. Three c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Dead in 45 minutes.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. antitoxic horse serum (PD 07173).[

G. P. No. 4350. Two c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 15 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{450}$ c. c. antitoxic horse serum (PD 08021).]

G. P. No. 4352. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{460}$ c. c. antitoxic horse serum (PD 08021).]

G. P. No. 4101. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{200}$ c. c. antitoxic horse serum (Alex. 190).]

G. P. No. 4511. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 35 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4512. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Dead in 81 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4623. One c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{300}$ c. c. antitoxic horse serum (Alex. 192).]

G. P. No. 4514. Two-tenths c. c. antitoxic horse serum (Natl. VIII. 17) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 4520. Two-tenths c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 65 minutes.

[Previous treatment: 42 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

G. P. No. 130. One-tenth c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Dead in 55 minutes.

[Previous treatment: 45 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 151. One-tenth c. c. antitoxic horse serum (Natl. IX. 19) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Stearns).]

G. P. No. 136. Do. Symptoms; recovered.

G. P. No. 144. Do. No symptoms.

G. P. No. 165. One hundredth c. c. antitoxic horse serum (Natl. IX. 17) inoculated subcutaneously. No symptoms.

3 hrs. 45 min. later, 0.1 c. c. Do. Symptoms; recovered.

[Previous treatment: 45 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Stearns 1351).]

G. P. No. 148. One-hundredth c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.

3 hrs. 45 min. later, 0.1 c. c. subcutaneously. Symptoms; recovered.

It will be seen from the above series that one-tenth of a cubic centimeter of horse serum into the peritoneal cavity of a susceptible guinea pig is sufficient to cause the death of the animal, whereas the same quantity subcutaneously causes symptons.

THE INFLUENCE OF LARGE QUANTITIES IN RENDERING GUINEA PIGS SUSCEPTIBLE.

The guinea pigs in the following series were first used as controls to show that the injection of large quantities of horse serum into the peritoneal cavity produces no effect. They were subsequently injected with horse serum again in large quantities into the peritoneal cavity to determine their susceptibility:

G. P. No. 296. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 14 days prior, 6 c. c. donkey serum injected into the peritoneal cavity.]

G. P. No. 297. Do.

G. P. No. 298. Same injection. No symptoms.

[Previous treatment: 14 days prior, 6 c. c. normal horse serum (Sp.) injected into the peritoneal cavity.]

G. P. No. 299. Do. Symptoms; recovered.

G. P. No. 300. Same injection. Symptoms; recovered.

[Previous treatment: 22 days prior, 6 c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity.]

G. P. No. 301. Do.

G. P. No. 302. Same injection. Symptoms; recovered.

[Previous treatment: 14 days prior 6 c. c. antitoxic horse serum (Natl. V. 14) injected into the peritoneal cavity.]

G. P. No. 303. Do. Symptoms (?)

G. P. No. 304. Same injection. Dead in 65 minutes.

[Previous treatment: 14 days prior 6 c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity.]

G. P. No. 305. Do. Dead in 95 minutes.

G. P. No. 306. Same injection. Symptoms; recovered.

[Previous treatment: 14 days prior, 3.5 c. c. antitoxic horse serum (NYBH 10B Gibson) injected into the peritoneal cavity.]

G. P. No. 307. Same injection. Dead in 48 minutes.

[Previous treatment: 14 days prior, 2 c. c. antitoxic horse serum (NYBH 13C Gibson) injected into the peritoneal cavity.]

G. P. No. 308. Same injection. Dead in 43 minutes.

[Previous treatment: 14 days prior, 3 c. c. antitoxic horse serum (Alex. A211) injected into the peritoneal cavity.]

G. P. No. 309. Same injection. Symptoms; recovered.

[Previous treatment: 14 days prior, 6 c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity.]

G. P. No. 310. Do. Symptoms; recovered.

It is plain, therefore, that guinea pigs may be rendered susceptible by the injection of large amounts of horse serum into the peritoneal cavity. We have seen, in another part of this bulletin, that these animals may also be rendered susceptible by the injection of large amounts into the subcutaneous tissue.

We believe, however, that guinea pigs are rendered more susceptible by the injection of the toxine-antitoxin mixture than by the horse serum alone.

Part VII.

SENSITIZING SUBSTANCE.

We already know that guinea pigs may be sensitized with either normal or antitoxic horse serum. It is of course both interesting and important to know whether the substance contained in horse serum which renders the guinea pigs sensitive is the same as the substance which exerts its poisonous action upon the second injection.

GUINEA PIGS MAY BE SENSITIZED WITH PRECIPITATED AND DIALYZED SERUM.

The following examples selected from our work demonstrate this point:

G. P. No. 4972. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 22 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{600}$ c. c. antitoxic horse serum (NYBH 13C, precipitated and dialyzed in accordance with Gibson's method).]

G. P. No. 4973. Do.

G. P. No. 4955. Six c. c. same serum. Dead in 27 minutes.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{450}$ c. c. antitoxic horse serum (NYBH 10B).]

G. P. No. 4957. Same injection. Symptoms; recovered.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. $5+\frac{1}{500}$ c. c. antitoxic horse serum (NYBH 10B).]

DRYING DOES NOT INJURE THE SENSITIZING SUBSTANCE.

A number of guinea pigs which were used by us in this work were sensitized with the antitoxic serum preserved under special conditions in this laboratory as the official standard in accordance with the law of July 1, 1902. This serum was reduced to dryness under special conditions and must be redissolved before using. Compare page 39.

The following are selected as experimental data proving that drying does not injure the sensitizing substance:

G. P. No. 6B. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Dead in 27 minutes.

[Previous treatment: 86 days prior inoculated subcutaneously with 0.22 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B25).]

G. P. No. 4488. Same injection. Dead in 10 minutes.

[Previous treatment: 12 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

SMALL QUANTITIES OF HORSE SERUM MAY RENDER GUINEA PIGS MORE SUSCEPTIBLE THAN LARGE QUANTITIES.

Before we learned that the antitoxin contained in the blood serum of the horse plays no rôle in rendering guinea pigs susceptible to subsequent inoculations of horse serum we planned and carried out an extensive series of experiments to determine just what relation the susceptibility of guinea pigs bears to the antitoxic potency of the horse serum injected. One series of guinea pigs was injected subcutaneously with one unit of an antitoxic horse serum containing 250 units to the c. c. This series, therefore, received $\frac{1}{250}$ c. c. of horse serum. The second series was treated subcutaneously with 250 units—that is, the guinea pigs received 1 c. c. of the horse serum. The third series of guinea pigs was treated with 1,000 units—that is, each guinea pig received 4 c. c. of the antitoxic horse serum. At varying intervals following these injections the pigs were given a second injection of horse serum in order to determine their susceptibility.

The following guinea pigs received $\frac{1}{250}$ c. c. antitoxic horse serum containing 1 unit:

G. P. No. 18. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity.

[Previous treatment: 1 day prior, inoculated subcutaneously with 1 unit, viz, $\frac{1}{250}$ c. c., horse serum (Natl. IX. 18).] No symptoms.

G. P. No. 17. Same injection as g. p. 18. No symptoms.

[Previous treatment: 4 days prior, as g. p. 18.]

G. P. No. 11. Same injection as g. p. 18. Dead in 35 minutes.

[Previous treatment: 17 days prior, as g. p. 18.]

G. P. No. 16. Same injection as g. p. 18. Symptoms; recovered.

[Previous treatment: 18 days prior, as g. p. 18.]

G. P. No. 15. Same injection as g. p. 18. Dead in 18 minutes.

[Previous treatment: 24 days prior, as g. p. 18.]

G. P. No. 19. Same injection as g. p. 18. Symptoms; recovered.

[Previous treatment: 32 days prior, as g. p. 18.]

G. P. No. 12. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into peritoneal cavity. Dead in 44 minutes.

[Previous treatment: 47 days prior, as g. p. 18.]

G. P. No. 10. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the pertioneal cavity. No symptoms.

[Previous treatment: 47 days prior, as g. p. 18.]

G. P. No. 13. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 68 days prior, as g. p. 18.]

The following guinea pigs received 1 c. c. antitoxic horse serum containing 250 units:

G. P. No. 29. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 1 day prior, inoculated subcutaneously with 250 units, viz, 1 c. c., horse serum (Natl. IX. 18).]

G. P. No. 27. Same injection as g. p. 29. No symptoms.
[Previous treatment: 4 days prior, as g. p. 29.]

G. P. No. 28. Same injection as g. p. 29. Symptoms; recovered.
[Previous treatment: 17 days prior, as g. p. 29.]

G. P. No. 26. Same injection as g. p. 29. Symptoms; recovered.
[Previous treatment: 18 days prior, as g. p. 29.]

G. P. No. 25. Same injection as g. p. 29. Symptoms; recovered.
[Previous treatment: 24 days prior, as g. p. 29.]

G. P. No. 23. Same injection as g. p. 29. Symptoms; recovered.
[Previous treatment: 32 days prior, as g. p. 29.]

G. P. No. 24. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Dead in 45 minutes.
[Previous treatment: 47 days prior, as g. p. 29.]

G. P. No. 22. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. Symptoms; recovered.
[Previous treatment: 47 days prior, as g. p. 29.]

G. P. No. 21. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 44 minutes.
[Previous treatment: 67 days prior, as g. p. 29.]

G. P. No. 20. Four c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.
[Previous treatment: 83 days prior, as g. p. 29.]

The following guinea pigs received 4 c. c. of antitoxic horse serum containing 1,000 units:

G. P. No. 38. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.
[Previous treatment: 1 day prior, inoculated subcutaneously with 1,000 units, viz, 4 c. c., antitoxic horse serum (Natl. IX. 18).]

G. P. No. 35. Same injection as g. p. 38. No symptoms.
[Previous treatment: 4 days prior, as g. p. 38.]

G. P. No. 36. Same injection as g. p. 38. Symptoms; recovered.
[Previous treatment: 17 days prior, as g. p. 38.]

G. P. No. 32. Same injection as g. p. 38. Symptoms; recovered.
[Previous treatment: 18 days prior, as g. p. 38.]

G. P. No. 34. Same injection as g. p. 38. Symptoms; recovered.
[Previous treatment: 24 days prior, as g. p. 38.]

G. P. No. 37. Same injection as g. p. 38. No symptoms.
[Previous treatment: 32 days prior, as g. p. 38.]

G. P. No. 39. Six c. c. antitoxic horse serum (Natl. IX. 17) injected into the peritoneal cavity. No symptoms.
[Previous treatment: 47 days prior, as g. p. 38.]

G. P. No. 31. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Symptoms; recovered.
[Previous treatment: 47 days prior, as g. p. 38.]

G. P. No. 33. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Dead in 46 minutes.
[Previous treatment: 68 days prior, as g. p. 38.]

G. P. No. 30. Four c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.
[Previous treatment: 83 days prior, as g. p. 38.]

We see from the above series that it required 17 days (or less) for the guinea pigs to become susceptible.

We learn from these three series that guinea pigs receiving 4 c. c. of antitoxic horse serum are apparently not quite so susceptible as those receiving the smaller amounts ($\frac{1}{250}$ c. c. and 1 c. c.); also, that those which received 1 c. c. are apparently not quite so susceptible as those which received only $\frac{1}{250}$ c. c. of the horse serum. From our work we know that it is the quantity of horse serum, and not the number of units contained in the serum, which plays the important rôle. If this relation of the amount of serum is true, we presume that animals receiving large quantities of horse serum for the first injection are less susceptible because they are rendered slightly immune, just as animals may be immunized against this action by daily repeated inoculations. When large quantities are given at the first injection, the horse serum is probably absorbed very slowly and acts somewhat in the same manner.

It would also seem from the above that guinea pigs are not rendered quite as susceptible by first injections of horse serum as by injections of the toxine-antitoxin mixture.

THE SENSITIZING SUBSTANCE IS NOT FREE IN THE BLOOD SERUM.

The following work seems to indicate that the substance which renders the guinea pigs susceptible to a second injection of horse serum is not free in the blood serum of the guinea pig in the same sense that the free receptors are in diphtheria antitoxic serum:

G. P. No. 298 (normal). Eight c. c. of a mixture containing 4 c. c. serum of susceptible guinea pigs and 4 c. c. normal horse serum, injected into the peritoneal cavity; the serums were mixed and injected at once. No symptoms.

G. P. No. 299. Ten c. c. above mixture injected into the peritoneal cavity. No symptoms.

G. P. No. 297. Five c. c. above mixture injected into the peritoneal cavity. No symptoms.

From the fact that the sensitizing substance does not appear to be free in the blood of the guinea pig and from the further fact that we were unable to transfer the immunity we might infer that this reaction may not reside so much in the blood serum as in the cells of the body.

THE EFFECT OF HEAT UPON THE SENSITIZING SUBSTANCE.

The following experiments plainly prove that the sensitizing substance is very resistant to heat, for guinea pigs previously treated with small quantities of horse serum heated to 60° C. for six hours subsequently proved highly susceptible:

G. P. No. 265. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 38 minutes.

[Previous treatment: 17 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (roan) heated to 60° C. for 6 hours.]

G. P. No. 266. Do. Dead in 20 minutes.

G. P. No. 267. Do. Dead in 25 minutes.

Part VIII.

ACTION OF HORSE SERUM UPON MAN AND OTHER ANIMALS.

MAN.

It may be that man can not be sensitized in the same way that we have shown is the case with guinea pigs. We made no human experiments, but have experimental data done by others which has a direct bearing on this question.

Pirquet and Schick injected children with antitoxic horse serum at intervals.

It has been customary to immunize numbers of children when exposed to diphtheria with antitoxic horse serum at intervals of from three to four weeks.

We have no doubt that there are many such instances on record and, so far as we know, this practice has never caused death.

Repeated injections of horse serum into man is not an infrequent occurrence. Patients suffering with diphtheria are often given injections of antitoxic serum at short and frequent intervals. It is also not rare for persons to have several attacks of diphtheria at long intervals and to be treated each time with antidiphtheric serum.

Certain serums, for example, the antitubercle serum of Maragliano, or the antirheumatic serum of Menzer are habitually used by giving injections at intervals of days or weeks.

In all of these cases of frequent and repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to this work. Pirquet and Schick in their work on Serumkrankeit give the following instances in which children received two injections of horse serum at intervals of from sixteen to forty-two days between the first and second injections.

Leopold H.—October 3, 1902. 100 c. c. scarlet-fever serum (Moser). Eight days following this injection symptoms of the serum disease appeared and lasted several weeks.

December 2, 1902. Fifty days after the first injection patient received 2 c. c. antidiphtheric serum under skin of arm.

In fifteen minutes following this second injection stormy symptoms set in. The boy began to cry and complained of nausea. Edema of the lip set in and soon spread over the whole face. In several hours general urticaria.

Heinrich K.—Three years old. June 13, 1902. 100 c. c. scarlet-fever serum. On the eighth day following the injection, symptoms of the serum disease appeared which lasted until the twenty-sixth day.

July 7. Thirty-four days following the first injection, 1 c. c. antidiphtheric serum injected into the left arm. The same afternoon urticaria and swelling of the lips. The next morning the arm was highly edematous.

Alexandrine K. Nine years old. May 28, 1902. 180 c. c. normal horse serum. Eleven days after the injection severe symptoms appeared, which lasted until June 17.

Sixteen days following the first injection, given 1 c. c. diphtheric antitoxic serum in the right forearm. Next morning, the hand swollen, very painful, and smartly to such an extent that the infection was considered of an erysipelatous nature. All redness and swelling disappeared in a few days.

Elizabeth K. Six and one-half years old. May 3, 1902. Given 180 c. c. scarlet-fever serum. May 15, ten days following, sudden rise of temperature, 39.4° C. Swelling of the lymph nodes. No exanthem.

Nineteen days after the first injection 50 c. c. scarlet-fever serum, following which occurred severe and painful edema of the skin of the abdomen, which spread to the labia and thighs, and disappeared in about a week.

Franz Z. June 6, 1903. 1 c. c. antidiphtheric serum. June 16 had urticaria, about nine days after the injection. August 2, eight weeks after first injection again given 5 c. c. antidiphtheric serum. Shortly following, urticaria and swelling of the face.

Frieda Z. June 10, 1903. 1 c. c. antidiphtheric serum. June 26, sixteen days following, the same injection repeated and acted in all respects similar to the above case, Franz Z., her brother. On August 2 she was again given 5 c. c. antidiphtheric serum. One hour later her face became red and swollen. The lids became so edematous that she could not open her right eye. In two hours there was general urticaria. Temperature, 38.6° C. Moderate swelling, locally.

Elli M. Four months old. First injection June 20, 1904. 12 c. c. antidiphtheric serum. Ten days later sudden rise of temperature 39° C. and urticaria. The fever lasted two days. Eruption later became measle-like and remained several days.

Nineteen days after the first injection 5 c. c. antidiphtheric serum. One hour after this severe general urticaria. From the site of injection, swelling of the skin of the abdomen. In the afternoon the temperature rose to 39.5° C.; frequent vomiting.

Leopoldine K. December 1, 1903. 5 c. c. horse serum. Twenty-seven days following the first injection, patient received 1 c. c. antidiphtheric serum. One hour following this last injection, definite swelling of the right hand, which later spread to the entire arm.

All these eight cases show this in common, that after the first injection of horse serum, the symptoms of the serum disease appear after the normal period of incubation, namely, between the eighth to thirteenth day. But when the same individuals are again injected with horse serum after intervals of sixteen to forty-two days, there reappears at once, or at least within twenty-four hours, symptoms of the serum disease.

Von Pirquet and Schick further give a list of 60 children who were injected with antitoxic horse serum at intervals from six days to seven and a half years between the first and second injections. They found that when the second injection was given from fourteen days to four months after the first injection they obtained, with great regularity, what they termed "the immediate reaction," but when the interval between the first and second injection is over four months they obtained little or no immediate reaction, but what they termed "an accelerated reaction," for the fever, urticaria, and other symptoms of the serum disease appeared on the fifth, sixth, seventh, or eighth day. It will be remembered that the normal period of incubation for the symptoms of the serum disease to appear after the first injection is between the eighth and thirteenth day. Von Pirquet and Schick lay special stress upon the phenomena of the "immediate" and "accelerated" reactions following the second injection.

We might also conclude despite the suggestion contained in Part X of our work upon sensitizing guinea pigs by feeding them with horse serum or horse meat that children may not be sensitized to the toxic action in horse serum by eating horse meat from the fact that horse meat is a favorite article of diet in certain European countries, and there is nothing on record to show that the injection of horse serum in those countries is fraught with more danger than where this practice does not obtain. We must, however, remember that our work has shown that guinea pigs are sensitized with exceedingly minute quantities of the strange proteid, and that repeated injections cause an immunity; and it is possible that the same action may be true of feeding.

Man reacts to the first injection of horse serum after a period of incubation of eight to thirteen days. Guinea pigs show practically no reaction following the first injection. Both react to a second injection. The reactions in man and the guinea pig, however, differ both in severity and kind. The relation, therefore, that our observations upon the guinea pig may have in its application to man must await further study. Of course, the fact that other animals besides man and guinea pigs react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action.

We have tested monkeys, rabbits, mice, dogs, cats, rats, chickens, and pigeons to determine whether any of these animals may be sensitized to the action of horse serum. Thus far we have obtained a response in the dogs, rabbits, and cats. This work is still in progress and will be reported at a future date.

Von Pirquet and Schick also found that the first injection into rabbits caused no clinical effect, but that subsequent subcutaneous injections caused immediate reaction in the production of local edema which extended even to gangrene. Second injections, when introduced intravenously, produced symptoms of collapse and even death.

Arthus also found that the injection of horse serum into rabbits caused no symptoms, whether the horse serum was injected subcutaneously or intravenously, but when he injected the serum every six days subcutaneously he obtained, after the fourth injection, edema and local reaction which continued to gangrene.

A rabbit which von Pirquet and Schick had previously treated with 8 injections was then given 2 c. c. of horse serum into the ear vein. The rabbit reacted to this in a minute and, after characteristic symptoms similar to those we see in guinea pigs, died in about four minutes.

Arthus obtained similar results with repeated injections of sterilized milk.

Part IX.

IMMUNITY.

ACTIVE IMMUNITY.

GUINEA PIGS MAY BE IMMUNIZED AGAINST THE TOXIC SUBSTANCE IN HORSE SERUM.

Guinea pigs may be immunized against this toxic substance, following the lines of active immunity produced by repeated inoculations of bacterial toxins. For instance, a guinea pig that has received an injection daily for ten days of small quantities (1 c. c.) of horse serum develops a high degree of immunity to subsequent injections of horse serum. Guinea pigs that have received more than ten inoculations on ten successive days develop a still higher degree of immunity.

G. P. No. 109. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 15 to 27 days prior, 10 subcutaneous inoculations, 1 c. c. each, of antitoxic horse serum (Natl. IX. 19).]

G. P. No. 111. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 36 to 48 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

G. P. No. 107. Six c. c. antitoxic horse serum (Natl. IX. 19) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 15 to 27 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

G. P. No. 106. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 36 to 48 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

G. P. No. 108. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Prior treatment: 37 to 50 days prior, 10 subcutaneous inoculations, 1 c. c. each, normal horse serum (Sam).]

6 days later, 1 c. c. normal horse serum (roan) inoculated subcutaneously on each of 12 successive days. No symptoms.

6 days later, 6 c. c. normal horse serum (roan) into peritoneal cavity. No symptoms.

G. P. No. 110. Six c. c. antitoxic horse serum (Natl. VIII. 18) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 to 50 days prior, 10 subcutaneous inoculations, 1 c. c. each, antitoxic horse serum (Natl. IX. 19).]

6 days later, 1 c. c. normal horse serum (roan) inoculated subcutaneously on each of 12 successive days. No symptoms.

6 days later, 6 c. c. normal horse serum (roan) into peritoneal cavity. No symptoms.

We noticed that the subcutaneous injection of horse serum into guinea pigs sometimes occasions a local reaction, as indicated by edema at the site of inoculation, but the above guinea pigs Nos. 108 and 110, which received a large number of injections, seemed to react less each time.

We give below a series of 11 guinea pigs injected with large quantities of horse serum at irregular intervals. It is plain from these experiments that a rather high degree of immunity may be induced by repeated injections.

G. P. No. 4101. One c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 37 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic horse serum (Alex. 190).]

8 days later, 1 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms, less severe.

16 days later, 6 c. c. into peritoneal cavity. No symptoms.

6 days later, 6 c. c. into peritoneal cavity. Symptoms.

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

G. P. No. 4530. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 10 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7+1 unit antitoxic horse serum (Standard B27).]

15 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms.

2 days later, 6 c. c. into peritoneal cavity. Restless.

6 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

9 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

33 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

21 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 4532. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 11 days prior, inoculated subcutaneously with 0.19 c. c. toxine. No. 7+1 unit antitoxic horse serum (Standard B27).]

14 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

8 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. Symptoms; recovered.

20 days later, 6 c. c. into peritoneal cavity. Symptoms; recovered.

G. P. No. 4432. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 7 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]

22 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

15 days later, 6 c. c. into peritoneal cavity. Symptoms.

33 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

21 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 4426. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 8 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]

27 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms (?).

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. into peritoneal cavity. Symptoms.

20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) in peritoneal cavity. No symptoms.

G. P. No. 4593 Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 3 days prior, inoculated subcutaneously with 0.001 c. c. toxine No. 7.]

18 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms.

8 days later, 6 c. c. into peritoneal cavity. Symptoms.

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. into peritoneal cavity. Symptoms.

20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

G. P. No. 4377. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 21 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{300}$ c. c. antitoxic horse serum (Natl. V. 7).]

Same day, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. Symptoms, less severe.

10 days later, 6 c. c. into peritoneal cavity. No symptoms.

2 days later, 6 c. c. into peritoneal cavity. No symptoms.

15 days later, 6 c. c. into peritoneal cavity. No symptoms.

34 days later, 6 c. c. into peritoneal cavity. No symptoms.

20 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

G. P. No. 4. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 27 days prior, inoculated subcutaneously with 6 c. c. antitoxic horse serum (Alex. 189).]

3 days later, 6 cc. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

19 days later, 6 c. c. into peritoneal cavity. Symptoms.

6 days later, 6 c. c. into peritoneal cavity. No symptoms.

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 4534. Six c. c. antitoxic horse serum (Natl. VIII. 17) injected into the peritoneal cavity. No symptoms.

[Previous treatment: 4 days prior, inoculated subcutaneously with 0.002 c. c. toxine No. 7.]

10 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

18 days later, 6 c. c. into peritoneal cavity. Symptoms (?).

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

33 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. No symptoms.

21 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 2. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. No symptoms.

[Previous treatment: None.]

3 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

19 days later, 6 c. c. into peritoneal cavity. Symptoms.

6 days later, 6 c. c. into peritoneal cavity. No symptoms.

43 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 18) into peritoneal cavity. Symptoms.

20 days later, 6 c. c. into peritoneal cavity. No symptoms.

G. P. No. 3. Ten c. c. normal horse serum (roan) injected into the peritoneal cavity. No symptoms.

[Previous treatment: None.]

2 days later, 6 c. c. same serum into peritoneal cavity. No symptoms.

3 days later, 6 c. c. antitoxic horse serum (Natl. VIII. 17) into peritoneal cavity. No symptoms.

9 days later, 6 c. c. into peritoneal cavity. No symptoms.

10 days later, 6 c. c. into peritoneal cavity. No symptoms.

PASSIVE IMMUNITY.

EFFECT OF NORMAL GUINEA-PIG BLOOD AND ORGANS UPON THE TOXICITY OF HORSE SERUM.

As a normal untreated guinea pig is entirely insusceptible to the toxic substances found in horse serum, it naturally occurred to us to try to ascertain whether the blood serum or the juices from the various organs of such a normal guinea pig had a restraining, neutralizing, or immunizing action when mixed with horse serum and the mixture inoculated into susceptible guinea pigs.

Normal G. P. A large, full-grown female guinea pig was chloroformed, then exsanguinated. Various organs, mentioned below, were minced and ground separately in a mortar with 10 c. c. of antitoxic horse serum (National VIII. 17). The mixtures were allowed to stand at room temperature 3 hours, then strained through cheese cloth, and injected into the peritoneal cavity of susceptible guinea pigs, as follows:

G. P. No. 4365. Six c. c. of a mixture containing equal parts of antitoxic horse serum and normal guinea-pig serum injected into the peritoneal cavity. Death in 40 minutes.

[Previous treatment: 31 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{350}$ c. c. antitoxic serum (PD&Co. No. 07173).]

G. P. No. 4463. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig liver injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic serum (Natl. VIII. 17).]

G. P. No. 4393. Seven c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig liver injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 28 days prior, inoculated subcutaneously with 0.2 c. c. toxine No. 59+1 unit antitoxic serum (Standard B27).]

G. P. No. 4470. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig spleen injected into the peritoneal cavity. No symptoms. For occasional irregularities see below.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic serum (Natl. IX. 17).]

6 c. c. antitoxic horse serum (Natl. VIII. 17) injected into peritoneal cavity of same pig one day later. Symptoms, recovered.

G. P. No. 4475. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig ovary injected into the peritoneal cavity. Death in 45 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic serum (Natl. IX. 18).]

G. P. No. 4466. Six c. c. of mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig kidney injected into the peritoneal cavity. Death in 45 minutes.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. antitoxic serum (Natl. VIII. 17).]

G. P. No. 4378. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig suprarenal injected into the peritoneal cavity. Death in 10 minutes.

[Previous treatment: 31 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{190}$ c. c. antitoxic serum (Natl. V. 10).]

G. P. No. 4464. Six c. c. of mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig thyroid injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic serum (Natl. VIII. 17).]

G. P. No. 4469. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. VIII. 17) and normal guinea-pig brain injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 26 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic serum (Natl. IX. 17).]

We may conclude from the above that neither the blood serum nor the organs of a normal guinea pig have any neutralizing action upon the toxicity of horse serum when injected into a sensitized guinea pig.

Guinea pig 4470, which received an injection of horse serum plus the spleen pulp of a normal guinea pig, showed no symptoms. We therefore repeated this experiment as follows:

G. P. No. 4493. Six c. c. of a mixture containing equal parts of antitoxic horse serum (Natl. IX. 19) and normal guinea-pig spleen injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 27 days prior, inoculated subcutaneously with 0.19 c. c. toxine No. 7 + 1 unit horse serum (Standard B27).]

We believe that occasional irregularities like this which have exceptionally occurred in this work may be accounted for by the fact that sometimes when the stomach and intestines are full and distended the inoculating needle enters the intestinal tract instead of the peritoneal cavity. While we suspected this possibility, it was proved to our satisfaction one day; after injecting a guinea pig, the intestinal contents flowed back into the barrel of the syringe, owing to accidental withdrawal of the piston. This particular guinea pig showed no symptoms as a result of the injection; whereas we knew it to be susceptible, because a subsequent operation, done a few hours later, caused characteristic symptoms.

For intraperitoneal inoculations the needle should be short and blunt to avoid this accident and the injection should be given over the region where the coils of small intestine lie close to the abdominal walls.

NEUTRALIZING EFFECT OF THE BLOOD AND ORGANS OF AN IMMUNIZED GUINEA PIG UPON THE TOXICITY OF HORSE SERUM.

We found that repeated injections of horse serum into a guinea pig produces a certain degree of immunity to the subsequent action of horse serum, and it naturally occurred to us to try whether such guinea pigs contained in their body juices an immunizing substance in sufficient quantity to neutralize the poisonous substance found in horse serum.

G. P. No. 109 (immunized). This guinea pig had received 10 subcutaneous inoculations of 1 c. c. each of antitoxic horse serum (Natl. IX. 19) on 10 successive days from Feb. 8 to 20; 15 days later, viz, March 7, it was inoculated with 6 c. c. antitoxic horse serum (Natl. IX. 19) into the peritoneal cavity. No symptoms.

Twenty-one days later, viz, March 28, it was again tested by injecting 6 c. c. of antitoxic horse serum (Natl. VIII. 18) into the peritoneal cavity. No symptoms.

As this guinea pig showed a high degree of resistance to the toxic action of horse serum it was killed two days later, March 30, with chloroform. About 15 c. c. of the blood was taken from the heart and defibrinated, the various organs were removed and placed in separate mortars and ground up with 10 c. c. of antitoxic horse serum (Natl. VIII. 18). This mixture of horse blood with organ juices of the guinea pig was allowed to stand about 3 hours at room temperature and strained through cheese cloth. It was then inoculated into the guinea pig.

G. P. No. 166. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *suprarenal* of g. p. 109 injected into the peritoneal cavity. Dead in 9 minutes.

[Previous treatment: 49 days prior, inoculated with 0.22 c. c. toxine No. $7+\frac{1}{300}$ c. c. horse serum (Stearns No. 1351).]

G. P. No. 158. Six c. c. same serum as g. p. 166 mixed with whole (defibrinated) *blood* of g. p. 109 injected into peritoneal cavity. Dead in 15 minutes.

[Previous treatment: As g. p. 166.]

G. P. No. 126. Six c. c. same serum as g. p. 166 mixed with *thyroid* of g. p. 109 injected into peritoneal cavity. Dead in 16 minutes.

[Previous treatment: As g. p. 166.]

G. P. No. 132. Six c. c. same serum as g. p. 166 mixed with *kidney* of g. p. 109 injected into peritoneal cavity. Symptoms, recovered.

[Previous treatment: As g. p. 166.]

G. P. No. 164. Six c. c. same serum as g. p. 166 mixed with *spleen* of g. p. 109 injected into peritoneal cavity. Symptoms, recovered.

[Previous treatment: As g. p. 166.]

G. P. No. 129. Six c. c. same serum as g. p. 166 mixed with *liver* of g. p. 109 injected into peritoneal cavity. No symptoms.

[Previous treatment: As g. p. 166.]

2 hr. 45 min. later. *To test susceptibility.* 6 c. c. same serum as g. p. 166 injected into peritoneal cavity. Dead in 30 minutes.

G. P. No. 147. Six c. c. same serum as g. p. 166 mixed with *brain* of g. p. 109 injected into peritoneal cavity. No symptoms.

[Previous treatment: As g. p. 166.]

1 hr. 20 min. later. *To test susceptibility.* 6 c. c. same serum as g. p. 166 injected into peritoneal cavity. Dead in 35 minutes.

G. P. No. 139 (control). Six c. c. same serum as g. p. 166 injected alone into peritoneal cavity. Dead in 15 minutes.

[Previous treatment: As g. p. 166.]

Owing to the fact that guinea pigs 129 and 147 showed no symptoms, the experiment was repeated, using organs from a similarly treated pig as follows:

G. P. No. 108 (immunized). This guinea pig had received 10 subcutaneous inoculations of 1 c. c. each of normal horse serum (Sam) on 10 successive days from Feb. 8 to 20; 37 days later, viz, March 29, it was inoculated with 6 c. c. antitoxic horse serum (Natl. VIII. 18) into the peritoneal cavity. Symptoms, recovered.

Beginning six days later, viz, April 4, and continuing 12 days, it was given a daily subcutaneous inoculation of 1 c. c. normal horse serum (roan). No symptoms.

Six days after the last inoculation the pig was given 6 c. c. normal horse serum (roan) into the peritoneal cavity. No symptoms.

This guinea pig, having showed a strong resistance to the toxic action of horse serum, was killed with chloroform and the brain, part of the spinal cord, and liver were minced and ground separately in a mortar with 20 c. c. antitoxic horse serum (Natl. VIII. 18). The mixture was allowed to stand about 3 hours at room temperature and strained through cheese cloth and then inoculated.

G. P. No. 4998. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *liver* of g. p. 108 injected into the peritoneal cavity. Dead in 95 minutes.

[Previous treatment: 39 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 + $\frac{1}{850}$ c. c. antitoxic horse serum (Wellcome 474A).]

G. P. No. 5044. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *brain* and portions of the *spinal cord* of g. p. 108 injected into the peritoneal cavity. Dead in 85 minutes.

[Previous treatment: 33 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5 + $\frac{1}{650}$ c. c. antitoxic horse serum (NYBH 13C).]

G. P. No. 252. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *brain* and portion of *spinal cord* of g. p. 108 injected into the peritoneal cavity. Symptoms, recovered.

[Previous treatment: 53 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (roan) heated to 60° C. for 30 min.]

G. P. No. 251. Six c. c. antitoxic horse serum (Natl. VIII. 18) mixed with *liver* of g. p. 108 injected into the peritoneal cavity. Symptoms; recovered.

[Previous treatment: 53 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (roan) heated to 60° C. for 30 min.]

As a further proof that the blood serum of an immunized guinea pig can not transfer this immunity, we added such serum from several guinea pigs immunized by repeated injections with normal horse serum; but when this mixture is injected into susceptible pigs they react in a characteristic manner. The experimental data upon this point will be seen in Part IV, page 37, upon the relation of precipitin to the toxic action.

It therefore appears that the "immune bodies," if such exist against the toxic action of horse serum, are not free in the blood and body juices, contrary to the case in diphtheria.

Part X.

FEEDING EXPERIMENTS.

GUINEA PIGS MAY BE SENSITIZED BY FEEDING HORSE SERUM.

Our work up to this point seemed to carry us very far away from an explanation of the cause of sudden death in man following the injection of horse serum. A thorough study of the literature upon this subject discloses the fact that almost all the unfortunate cases of this kind have been due to the first injection of horse serum. If, therefore, man may be sensitized to a toxic principle in horse serum, it must be by some other means than a previous injection of horse serum. We have seen that von Pirquet and Schick, in their studies on serum disease, have actually injected children at intervals of several weeks without any such effect as we have found upon guinea pigs. Further, that on numerous occasions children have been given successive injections of diphtheria antitoxin at intervals of three or four weeks for purposes of immunization.

In casting about for another explanation it occurred to us that probably this action may take place through eating horse meat. We therefore carried on a number of feeding experiments upon guinea pigs. We first fed guinea pigs with normal horse serum by means of a stomach tube, with the following results:

G. P. No. 116. One feeding of 5 c. c. normal horse serum (Jane) into stomach.
27 days later inoculated with 6 c. c. antitoxic horse serum (Natl. IX. 19) into the peritoneal cavity. No symptoms.

G. P. No. 114. Four daily feedings, each of 5 c. c., same serum.
27 days after first feeding and 23 after last, same inoculation as g. p. 116. No symptoms.

G. P. No. 112. Five daily feedings, 5 c. c. each, same serum.
27 days after first feeding and 22 after last, same inoculation as g. p. 116. No symptoms.

G. P. No. 113. Six daily feedings, 5 c. c. each, same serum.
27 days after first feeding and 21 after last, same inoculation as g. p. 116. No symptoms.

G. P. No. 115. Ten daily feedings, 5 c. c. each, same serum.
27 days after first feeding and 15 after last, same inoculation as g. p. 116. Symptoms, recovered.

Only one of these five pigs showed definite and characteristic symptoms following the intrastomachal administration of the horse serum. This pig, No. 115, which was sensitive to the subsequent injections of horse serum, had received ten feedings of 5 c. c. each of normal horse serum daily. In view of the fact, however, that exceedingly small quantities ($\frac{1}{1000000}$ c. c.) of horse serum is sometimes sufficient to sensitize a guinea pig, it seemed to us that feeding animals with a stomach tube might cause slight abrasions, resulting in absorption, so that pigs fed in this manner might really be sensitized through minute wounds of the mucous membrane.

We therefore used another series of pigs and fed the animals without using artificial means. As guinea pigs will not drink horse serum, we found it necessary to dry the serum, reduce it to powder, and then mix it with ground carrots. In this way they took the serum unknowingly with their food. By this means definite amounts of the horse serum may be given and the quantity given to each animal accurately controlled. Half a gram of the dried normal horse serum was thus fed to each pig, which was afterwards tested for susceptibility, as follows:

G. P. No. 4551. Fed 0.5 gm. dried normal horse serum daily from March 15 to 28. Injected March 28 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX. 19), 13 days from first and 0 days from last feeding. Symptoms; recovered.

G. P. No. 245. Fed 0.5 gm. dried normal horse serum daily from March 15 to 28. Injected March 28 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX. 18), 13 days from first and 0 days from last feeding. No symptoms.

G. P. No. 4581. Fed 0.5 gm. dried normal horse serum daily from March 15 to 29. Injected April 5 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 7 days from last feeding. Symptoms; recovered.

G. P. No. 4540. Fed 0.5 gm. dried normal horse serum daily from March 15 to 31. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 6 days from last feeding. Symptoms; recovered.

G. P. No. 4546. Do.

G. P. No. 4575. Fed 0.5 gm. dried normal horse serum daily from March 15 to April 6. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 0 days from last feeding. Symptoms; recovered.

G. P. No. 172. Fed 0.5 gm. dried normal horse serum daily from March 15 to April 5. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 21 days from first and 1 day from last feeding. Symptoms; recovered.

G. P. No. 173. Do.

G. P. No. 276. Fed 0.5 gm. dried normal horse serum daily from March 15 to April 9. Injected April 9 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII. 18), 25 days from first and 0 days from last feeding. Dead in 55 min.

There is no doubt from the above experiments that guinea pigs may be sensitized by feeding to them horse serum. Of the nine pigs so fed in the above series eight reacted to subsequent injections of horse serum, one of these died.

These remarkable results are not surprising when we remember that Uhlenhuth [a] showed the possibility of the intrastomachal immunization in working on antiproteid immunization. He fed rabbits daily for a number of weeks with egg albumin by means of a stomach tube. The rabbits' serum was examined every eight days. After twenty-four days it developed the power to precipitate egg albumin.

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[a] Uhlenhuth, —: Neuer Beitrag zum spezifischen Nachweis von Eiereiweiss auf biologischem Wege. Deut. med. Woch., Bd. 26, 1900, p. 734–735.

Metalnikoff [a] reports that he attained immunization by feeding one animal with the blood of another. He fed horse's blood to white rats. After three or four feedings no agglutination or hemolytic action of the rats' blood upon the horse's corpuscles was noted. In one week the action appeared feeble and increased to decided strength after one or two months.

GUINEA PIGS MAY BE SENSITIZED BY FEEDING HORSE MEAT.

After demonstrating the possibility of rendering guinea pigs susceptible by feeding them with horse serum, we next fed a series with horse meat. The following series was fed with indefinite amounts of horse meat, both fresh and dried ground up with carrots, as guinea pigs do not like fresh horse meat and must be very hungry in order to eat it.

G. P. No. 242. Fed 9 days on horse meat from Feb. 15. Injected March 9 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX, 17), 21 days after first and 15 days after last feeding. Symptoms; recovered.

G. P. No. 237. Fed 9 days on horse meat from Feb. 15. Injected March 10 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. IX, 17), 25 days after first and 16 days after last feeding. Symptoms; recovered.

G. P. No. 238. Do.

G. P. No. 239. Do.

G. P. No. 240. Do.

G. P. No. 244. Do.

G. P. No. 241. Fed 9 days on horse meat from Feb. 15. Injected April 5 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 49 days after first and 40 days after last feeding. Symptoms?; recovered.

G. P. No. 243. Fed 9 days on horse meat from Feb. 15. Injected March 13 into peritoneal cavity with 10 c. c. aqueous extract dry horse meat, 26 days after first and 17 days after last feeding. No symptoms.

As the above series was fed with indefinite amounts, another series of guinea pigs was placed upon definite weighed amounts of horse meat. Four grams of the dried ground horse meat were mixed with fresh carrots and fed to the pigs daily. Almost all the pigs placed upon this diet did poorly, their coats became shaggy and the animals lost weight.

G. P. No. 246. Fed 4 gm. dried horse meat daily from March 15 to 28. Injected March 28 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 13 days after first and 0 day after last feeding. Symptoms?; recovered.

G. P. No. 4562. Do.

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[a] Metalnikoff, —: Ueber hamolytisches Serum durch Blutfütterung. Centblt. f. Bakt., v. 29, 1901, p. 531–533.

G. P. No. 4544. Fed 4 gm. dried horse meat daily from March 15 to 29. Injected April 5 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 7 days after last feeding. Symptoms; recovered.

G. P. No. 4543. Fed 4 gm. dried horse meat daily from March 15 to April 6. Injected April 6 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 0 day after last feeding. No symptoms.

G. P. No. 4563. Do. Symptoms?; recovered.

G. P. No. 4371. Do. No symptoms.

G. P. No. 270. Do. Symptoms; recovered.

G. P. No. 4549. Do. No symptoms.

G. P. No. 4538. Fed 4 gm. dried horse meat daily from March 15 to 31. Injected April 6 with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 6 days after last feeding. Symptoms; recovered.

G. P. No. 174. Do. Dead in 20 minutes.

G. P. No. 275. Fed 4 gm. dried horse meat daily from March 15 to April 9. Injected April 9 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 25 days after first and 0 days after last feeding. No symptoms.

G. P. No. 4557. Do.

G. P. No. 4574. Do. Symptoms?; recovered.

G. P. No. 4569. Do. No symptoms.

G. P. No. 4566. Do. Symptoms; recovered.

It is perfectly plain from the above that guinea pigs may be sensitized by feeding them horse meat as well as horse serum.

MAY GUINEA PIGS BE SENSITIZED BY FEEDING BEEF?

Another series of experiments was then conducted by feeding guinea pigs with beef. The meat was dried, ground, and mixed in definite weighed amounts with ground carrots.

G. P. No. 247. Fed 4 gm. dried beef daily from March 15 to 28. Injected March 28 into peritoneal cavity with 5 c. c. antitoxic horse serum (Natl. VIII, 18), 13 days after first and 0 day after last feeding. Symptoms?; recovered.

G. P. No. 269. Fed 4 gm. dried beef daily from March 15 to 29. Injected April 5 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 7 days after last feeding. Symptoms?; recovered.

G. P. No. 171. Fed 4 gm. dried beef daily from March 15 to April 6. Injected April 6 into peritoneal cavity with 6 c. c. antitoxic horse serum (Natl. VIII, 18), 21 days after first and 0 day after last feeding. No symptoms.

Five guinea pigs were placed in this series, but two of them died, apparently as a result of the diet.

We conclude, as a result of these feeding experiments, that guinea pigs may be highly sensitized to the toxic action of horse serum by previous feeding with horse serum or horseflesh. So far as conclusions may be drawn from the few experiments done upon feeding guinea pigs with beef, we feel justified in stating that guinea pigs may also be rendered slightly susceptible in this way. Whether pigs fed with beef would be more susceptible subsequently to the injection of cattle serum than horse serum is reserved for further study.

The fact that guinea pigs may be rendered susceptible by the feeding of strange proteid matter opens an interesting question as to whether sensitized guinea pigs may also be poisoned by feeding with the same serum. If man can be sensitized in a similar way by the eating of certain proteid substances it may throw light upon those interesting and obscure cases in which the eating of fish and other sea food, etc., by certain individuals habitually causes sudden and sometimes serious symptoms.

Part XI.

HEREDITARY TRANSMISSION OF THE SUSCEPTIBILITY IN GUINEA PIGS.

So far as may be judged from the following data on this subject the susceptibility to the toxic effects of horse serum may be transmitted from the mother guinea pig to her young:

FAMILY NO. 1.

Mother g. p. No. 105. One c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

[Previous treatment: 55 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. normal horse serum (Jane).]

The progeny of the above guinea pig, when about five days old, was tested as follows:

G. P. No. 105A. One c. c. normal horse serum (roan) inoculated subcutaneously. Symptoms; recovered.

G. P. No. 105B. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms; recovered.

FAMILY NO. 2.

Mother g. p. No. 66. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Dead in 120 minutes.

[Previous treatment: 85 days prior, 6 c. c. normal horse serum (Sam) injected into the peritoneal cavity.]

The progeny of the above guinea pig, when about a week old, was tested as follows:

G. P. No. 66A. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

G. P. No. 66B. Do.

FAMILY NO. 3.

Mother g. p. No. 103. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

[Previous treatment: 71 days prior, $\frac{1}{250}$ c. c. normal horse serum (Jane) inoculated subcutaneously; 33 days later 6 c. c. antitoxic horse serum (Natl. IX, 17) injected into the peritoneal cavity. Symptoms.]

The progeny of the above guinea pig, when about ten days old, was tested as follows:

G. P. No. 103A. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.

FAMILY NO. 4.

Mother g. p. No. 4463. Six c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptons (?).

[Previous treatment: 99 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7 + $\frac{1}{200}$ c. c. antitoxic horse serum (Natl. VIII, 17). 26 days later, 6 c. c. antitoxic horse serum (Natl. VIII, 17) + liver pulp of normal guinea pig injected into the peritoneal cavity. Symptoms.]

The progeny of the above guinea pig, when about ten days old, was tested as follows:

G. P. No. 4463A. One c. c. normal horse serum (roan) injected into the peritoneal cavity. Symptoms.
G. P. No. 4463B. Do.

FAMILY NO. 5.

Mother g. p. No. 14. Six c. c. normal horse serum (roan) injected into the peritoneal cavity Symptoms; recovered.

[Previous treatment: 94 days prior, inoculated subcutaneously with $\frac{1}{250}$ c. c. antitoxic horse serum (Natl. IX, 18.)]

The progeny of the above guinea pig, when five days old, was tested as follows:

G.P. No. 14A. One c. c. normal horse serum (roan) injected into the peritoneal cavity Dead in 30 minutes.
G. P. 14B. Do.
G. P. No. 14C. Do. Dead in 35 minutes.

It is plain from the above that young guinea pigs born of a susceptible mother are themselves susceptible, often more so than the mother herself.

To control the effect of horse serum upon such young guinea pigs the following data are given:

(All controls born of normal parents.)

Control g. p. No. 01. One c. c. normal horse serum (roan) inoculated subcutaneously when 5 days old. No symptoms.
Control g. p. No. 02. Two c. c. same serum when 1 week old. No symptoms.
Control g. p. No. 03. Same injection when 6 days old. No symptoms.
Control g. p. No. 04. Same injection when 4 days old. No symptoms.

These four controls were selected from four different litters.

These results upon the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea pigs may throw light upon the well-known inherited tendency to tuberculosis in children born of a tuberculous parent.

There are certain analogies between the action of tuberculosis and horse serum. Both produce a hypersensitiveness and also a certain degree of immunity. Now that we have proved that this hypersensitiveness or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea pigs, may it not throw light upon the fact that tuberculosis "runs in families?" While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young this is, as far as we know, the first recorded instance in which hypersensitiveness, or anaphylaxis, has been experimentally shown to be hereditarily transmitted from a mother to her young.

Part XII.

TOXIC ACTION OF OTHER ALBUMINOUS SUBSTANCES.

As we found that guinea pigs may be so highly sensitized to the toxic action of horse serum and to a certain extent the serum from other animals we made a few experiments to determine whether other albuminous substances possessed the same toxic properties under these conditions. For this purpose we used egg albumin, peptone, skimmed milk, hemoglobin and vegetable proteid.

G. P. No. 4366. Six c. c. of a saturated solution of egg albumin were injected into the peritoneal cavity. No symptoms.

[Previous treatment: 30 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. 7+$\frac{1}{400}$ c. c. horse serum (PD & Co. 08022–A)].

Two days later this guinea pig was tested for susceptibility by the inoculation of 6 c. c. antitoxic horse serum (Natl. VIII. 17) into the peritoneal cavity. Dead in 50 minutes.

G. P. No. 4924. Six c. c. of a saturated solution of peptone injected into the peritoneal cavity. No symptoms.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{300}$ c. c. antitoxic horse serum (Cutter 1540)].

Three days later this guinea pig was tested for susceptibility by the inoculation of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Symptoms; recovered.

G. P. No. 4926. Six c. c. skimmed milk injected into the peritoneal cavity. No symptoms.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{460}$ c. c. antitoxic horse serum (Cutter 1540)].

Three days later tested for susceptibility by the injection of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Dead in 11 minutes.

G. P. No. 4919. Ten c. c. vegetable proteid from oats injected into the peritoneal cavity. No symptoms.

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{280}$ c. c. antitoxic horse serum (Stearns 16 H)].

Three days later tested for susceptibility by the injection of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Dead in 25 minutes.

G. P. No. 4925. Six c. c. hemoglobin solution in water from washed normal (roan horse) corpuscles injected into the peritoneal cavity. No symptoms (?)

[Previous treatment: 40 days prior, inoculated subcutaneously with 0.139 c. c. toxine No. 5+$\frac{1}{370}$ c. c. antitoxic horse serum (Cutter 1540)].

Three days later tested for susceptibility by the injection of 6 c. c. antitoxic horse serum (Natl. IX. 17) into the peritoneal cavity. Symptoms; recovered.

G. P. (normal). Four and a half c. c. solution of hemoglobin (watery solution of washed red blood corpuscles of roan horse) injected into peritoneal cavity. No symptoms.

G. P. No. 4989. Ten c. c. same injection. Symptoms (?)

[Previous treatment: 25 days prior, inoculated subcutaneously with 0.22 c. c. toxine No. $7+\frac{1}{200}$ c. c. antitoxic horse serum (Alex. A202)].

To control the two preceding pigs a solution of hemoglobin, obtained by dissolving red blood corpuscles from a normal horse (roan), was injected into the peritoneal cavity of a normal guinea pig, with negative results.

Whether guinea pigs treated with these albuminous substances are rendered susceptible to subsequent injections of the same albumin is now being studied.

Part XIII.

A REVIEW OF THE LITERATURE RELATING TO OUR WORK.

We feel it incumbent upon us to compare our results with the work of others and, therefore, have introduced this discussion upon the toxic action of serums in general, especially in relation to the production of immunity and hypersensitiveness.

NOTE.—In this historical development we have drawn largely upon the splendid review of the literature as given by Uhlenhuth in "Zur Kenntniss der giftigen Eigenschaften des Blutserums" (Zeit. f. Hyg., v. 26, 1897), and C. Frh. von Pirquet and B. Schick in "Die Serumkrankheit" (K. K. Universitäts-Kinder-Klinik, Wien, 1905).

Transfusion was formerly used as a therapeutic measure. The first reference to this practice is June 15, 1667, when von Denis transfused lamb's blood (cited in Landois's article on transfusion, Eulenburg's Realenzyklopädie, 3. Auflage). This practice gradually fell into disuse but was again revived about the beginning of the last century, when transfusion was used for acute anemia and poisonings.

The intravenous injection of lamb's blood was soon shown to be associated with great danger. High fever in half an hour, emboli, hemorrhages, hemoglobinuria, etc., were often noticed. The causes of these serious symptoms, as well as the scientific basis of our knowledge upon the effects of transfusion, were first made clear by the work of Landois and Ponfick in the years 1873 and 1875. These authors showed that the blood of alien species caused solution of the corpuscles when brought into the circulation. Naunyn and Francken showed that the injection of dissolved hemoglobin into the circulation caused coagulation. Great numbers of the white corpuscles were so acted upon by the dissolved hemoglobin that the fibrin-forming elements were thus set free, resulting in the formation of clots within the vessels. When this coagulation is extensive, death may result from asphyxia.

Dallera [a] in 1874 reported that urticarial eruptions may follow the transfusion of blood.

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[a] Dallera: Considerazioni e casi clinici di transfusione del sangue. Il morgagni, 1874, 7.

Neudörfer[a] refers to the fact that urticaria frequently follows transfusion.

Landois[b] also refers to this complication.

In consequence of the severe injury which often resulted from the transfusion of lamb's blood the practice was entirely discontinued. Recently, however, Dominici[c] has again taken up the subject.

In England at one time the transfusion of milk was practiced, but proved dangerous and without benefit, as stated by Montard, Martin, and Richet. (Injections intraveineuses de lait et de sucre, Soc. de Biol., 1879.)

Latterly the transfusion of lamb's blood has given place to physiological salt solution and also to the transfusion of defibrinated human blood.

In the year 1894 the use of diphtheria antitoxin introduced the widespread practice of injecting horse serum. This practice differed from the previous work with transfusion in that the injections are generally not given intravenously, but subcutaneously, and the quantity of the alien albuminous substance is relatively small. According to von Pirquet and Schick this accounts partly for the fact that in the majority of these injections no injurious consequences followed. Luebinski (Über eine Nachwirkung des Antitoxins bei Behandlung des Diphtherie, Deut. med. Woch., 1894) was the first to call attention to a case of exanthematous eruption following the injection of antitoxin serum. Soon afterwards Schütz,[d] Cnyrim,[e] Asch,[f] Rembold,[g] and Treymann[h] reported urticarial eruptions following the prophylactic injection of antitoxic horse serum. Following these came a great mass of evidence which made it clear that following the injection of antidiphtheric serum these sequelæ were comparatively harmless.

In relation to the cause of these symptoms it was generally held that the serum contained some toxic substance, probably a globulin. This explanation led manufacturers to the commendable practice of starving their horses about twenty-four hours prior to bleeding, in order to eliminate as far as practicable the freshly assimilated products of digestion.

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[a] Neudörfer: Beiträge zur Bluttransfusion. Zeitschr. f. Chirurgie, Bd. 6.

[b] Landois: Die Transfusion des Blutes, Leipzig, 1875. ——: Transfusion. Eulenburg's Realencyclopädie, 3.Aufl., 1900.

[c] Dominici: Transfusion. Wiener med. Wochenschr., 1895.

[d] Schütz: Discussionsbemerkung zu Piorkowski's Vorträge. Berl. med. Gesellsch., 1901.

[e] Cnyrim: Zwei Fälle von Erkrankungen nach Anwendung des Diphtherieserums. Deut. med. Woch., 1894, p. 898.

[f] Asch: Zur Casuistik der Heilserumexantheme. Berl. klin. Woch., 1894.

[g] Rembold: Zwei Fälle von Erkrankung nach Anwendung des Heilserums. Deut. med. Woch., 1894, p. 963.

[h] Treymann, Otto: Ein Fall von acuter hämorrhagischer Nephritis nach Anwendung des Behring'schen Diphtherieheilserums. Deut. med. Woch., 1894, p. 952.

Heubner [a] and von Bókay [b] expressed the opinion that these manifestations were due to other properties than the antitoxin in the serum.

Johannessen [c] believed that the agent in horse serum which produced these symptoms was due to the introduction into the body of an alien serum, for he produced the same effects by injecting normal horse serum. In 22 cases fever resulted, and skin eruptions in 12.

Sévestre [d] referred the exanthems which appear in eight to ten days back to the serum which had been injected. However, he thought that the measle-like eruption and fever was the expression of a streptococcus infection. Netter, however, in the discussion, believed these symptoms to be caused by the serum.

Almost all authors express the opinion that the symptoms caused by the injection of serum are of minor consequence. The fact that from time to time serious symptoms and even death followed the injection of diphtheria antitoxic serum does not alter the general impression that as a rule such injections are harmless. *The benefits far outweigh the danger.*

Gottstein,[e] in addition to the Langerhans case, collected 8 deaths following the injection of serum in those having diphtheria and 4 in those not sick with diphtheria.

We have collected from the literature 19 cases of such unfortunate results, and know personally of several more which have not been reported. Von Pirquet and Schick express the opinion that a critical examination of these cases may be otherwise explained, and authors generally have not permitted this rare accident to counterbalance the great benefit possible from the use of diphtheria antitoxic serum.

It seems to have been definitely shown that the skin eruptions and other symptoms following the injection of horse serum follow in direct proportion to the amount of serum injected. This has resulted in endeavors to concentrate the serum as much as possible.

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[a] Heubner: Praktische Winke zur Behandlung der Diphtherie mit Heilserum. Deut. med. Woch., 1894, p. 701. —— Klinische Studien über die Behandlung der Diphtherie mit dem Behring'schen Heilserum. Leipzig, 1895. —— Über die Anwendung des Heilserums bei der Diphtherie. Jahrb. f. Kdheilk., Bd. 38, p. 231.

[b] Von Bókay: Die Heilserumbehandlung gegen Diphtherie im Budapeste Stefanie-Kinderspital. H. f. Kdh., Bd. 44, 1897. —— Meine Erfolge mit Behring's Diptherieheilserum. Deut. med. Woch., 1895, p. 233. —— Meine Erfahrungen mit dem Moser'schen polyvalenten Scharlach-Streptokokkenserum. Deut. med. Woch., 1904.

[c] Johannessen: Über Injektionen mit antidiphtherischen Serum und reinem Pferdeserum. Deut. med. Woch., 1895. —— Über Immunisierung bei Diphtherie. Deut. med. Woch., 1895.

[d] Sévestre: Des accidents causés par le streptococque à la suite des injections de sérum de Roux. Bull. de la Soc. méd. des hôpitéaux, July 19, 1895, Jan. 31, 1896. La méd. moderne, 1896.

[e] Gottstein: Über Todesfälle, welche bei der Anwendung des Diphtherieheilserums beobachtet worden sind. Therap. Monatsh., 1896.

Bujwid [a] believed that these manifestations were due largely to fresh horse serum, and favors the use of old seasoned antitoxin for therapeutic purposes.

Hamburger and Moro [b] have shown that the injection of alien serums into man result in the formation of precipitins.

Hamburger,[c] in a further work, drew a distinction between alien albuminous substances introduced through the digestive tract and by other channels.

Upon the general toxic properties of blood we quote the following instances, taken largely from Uhlenhuth: [d]

Stern,[e] 1893, found that the blood from a case of erysipelas in amounts of 0.5 to 1 c. c. killed white mice, while normal human blood required 3 c. c. or more to kill the animals.

Rummo,[f] 1891, showed that blood serum from cases of typhoid fever, malaria, pneumonia, and eclampsia had a high grade of toxicity for rabbits when injected intravenously.

Albu,[g] 1897, confirm Rummo's experiments, and found an increase of toxicity in persons suffering from pneumonia, severe chronic bronchitis, epilepsy, uræmia, and puerperal sepsis.

Upon the toxicity of the blood serum of man in health and disease further work has been done by Charrin,[h] Leclainche and Rémond,[i] Chambrelent and Tarnier, [j] Ludwig and Savor,[k]Guinard and Dumarest,[l]

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[a] Bujwid, O.: Kann des Antidiphtherieserum schädlich sein? Polnisch, ref. Virchow's Jarhb., 1897, II, p. 659.

[b] Hamburger, F., and Moro: Uber die biologischen nachweisbaren Verändertungen des menschlichen Blutes nach Seruminjektion. Wiener klin. Woch., 1903.

[c] Hamburger, F.: Zur Frage der Immunisierung gegen Eiweiss. Wiener klin. Woch., 1902. ——: Arteigenheit und Assimilation. Wien, 1903.

[d] Uhlenhuth: Zur Kenntniss der giftigen Eigenschaften des Blutserums. Zeit. f. Hyg., vol. 26, 1897, p. 384.

[e] Stern: Ueber einige Beziehungen zwischen menschlichen Blutserum und pathogenen Bakterien. (XII Congress für innere Medicin, Wiesbaden, 1893): Deut. med. Woch., 1893.

[f] Rummo: Ueber die Giftigkeit des Blutserums bei Menschen und Thieren im normalen Zustande und bei Infectionskrankheiten. Wiener med. Woch., 1891.

[g] Albu: Untersuchungen über die Toxicität normaler u. pathol. Serumflüssigkeit. Virchow's Archiv., bd. 140, 1897.

[h] Charrin: Compt. rend. soc. biol., 1890.

[i] Leclainche and Rémond: Compt. rend. soc. biol., 1890, p. 1037.

[j] Ludwig and Savor: Monatschrift für Geburtshulfe, bd. 1, 1895.

[k] Tarnier and Chambrelent: Note relative à la recherche de la toxicité du sérum sanguin dans deux cas d'eclampsie puerpérale. Soc. de biolog., 1892.

[l] Guinard and Dumarest: Note sur la détermination de la toxicité du serum sanguin: Techniques et résultats. Compt. rend., 1897, No. 15. Variations de la toxicité du sérum sanguin dans certaines infections expérimentales. Loc. cit., No. 18. A propos de la détermination physiol. et clinique de la toxicité du sérum humain. Loc. cit.. No. 18.

Bar and Rénon,[a] Picolini and Conti,[b] Mairet and Bosc.[c] All of these authors determined the toxicity of the blood serum in accordance with Rummo's method of intravenous injections into rabbits.

The poisonous properties of the blood serum from healthy men, according to the work of these authors, varies considerably, as may be judged from the following summary:

Rummo found 10 c. c. as the fatal dose for 1 kilogram of rabbit.

Lugwig and Savor, 8 to 9 c. c.

Chambrelent and Tarnier, 10 c. c.

Albu, 9.5 to 11 c. c.

Mairet and Bosc, 12.5 to 18.0 c. c.

Guinard and Dumarest, 17 c. c.

Leclainche and Rémond, 23 c. c.

Charrin, 27 c. c.

It will therefore be seen that the fatal dose of normal human serum, according to these different investigations, varies between 8 c. c. and 27 c. c. per kilogram of rabbit.

Uhlenhuth found that the intravenous injection of from 7 to 10 c. c. of normal blood serum per kilogram of rabbit regularly caused death within one-half hour.

The blood serum of other animals was injected intravenously into rabbits and showed considerable differences in their toxicity. The quantity necessary to kill in proportion of 1 kilogram of rabbit was:

	c.c.
Sheep serum	11
Hog serum	12
Cattle serum	6

Horse serum on the other hand, when injected in such large quantities as 60 c. c. and more per 1 kilogram of rabbit, not only did not kill but caused no reaction at all.

In 1888 Mosso [d] discovered that eel serum in doses as small as 0.02 c. c. per 1 kilogram of rabbit caused death.

Uhlenhuth, finding intravenous injections into rabbits unsatisfactory, used subcutaneous inoculations into guinea pigs. He found that the injection of small quantities (0.5 c. c.) of human, sheep, hog, and rabbit serums caused infiltration, and of large quantities (15 to 20 c. c.) necrosis. On the other hand, cattle serum caused death in doses of 10 to 15 c. c., while horse serum in doses of 20 c. c. caused no infiltration and in larger doses a very slight infiltration which entirely disappeared within forty-eight hours.

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[a] Bar and Rénon: De la toxicité du sang et de l'urine chez une femme atheinte de troubles gravido-cardiagues. Compt. rend. soc. biol., 1894.

[b] Picolini and Conti: Toxicité du sérum sanguin et de l'urine dans la pneumonie.

[c] Mairet and Bosc: Recherches sur les causes de la toxicité du sérum du sang. Compt. rend., v. 119, p. 292. Toxicité du sérum du sang de l'homme sain. Comp. rend., 1897. *Des effects de la chaleur sur la toxicité du sérum. Compt. rend., 1894.

[d] Mosso: Die giftige wirkung des serums der muraeniden. Arch. f. exper. pathol. u. pharmakol., bd. 25, p. 111.

Pfeiffer [a] confirmed Uhlenhuth's findings and believed that the necrotic action was due to a "haptin" substance identical with the hemolytic action of blood serum.

Von Pirquet and Schick, in their work on "Die Serumkrankheit," have developed the clinical manifestations following the injection of horse serum in man into a syndrome which they call "the serum disease." The principal manifestations of this disease are a period of incubation varying from eight to thirteen days, fever, skin eruptions, swelling of the lymph glands, leukonemia, joint symptoms, edema and albuminuria.

The following historical development of this phase of the subject, which bears such a close relation to our work, is largely a free translation of von Pirquet and Schick's monograph.

As a result of their studies von Pirquet and Schick consider their most important conclusions to be the fact that the power of reaction in serum disease may show itself either immediately or after a shortened period of incubation.

Arthus [b] described hypersensitiveness and explained its significance with special reference to the serum disease. He based his work upon that of Richet,[c] who showed in his experiments upon actinia poison (the actinia is a sea anemone) that the first injection causes death in dogs in doses between 0.2 and 0.18 gram per kilogram of body weight. In no case does death occur before ten hours. Upon subsequent injection the animal died from smaller doses (0.15 gm.) and much more quickly. These injections were all intravenous. The following is a characteristic example: A dog receives the first time 0.1 gm. of the actinia poison per kilogram of his body weight, from which it shows scarcely perceptible symptoms. After twenty-two days the dog was again injected with 0.1 gm. per kilogram of weight in the saphenous vein. Several seconds later the dog developed coughing respiration and could hardly drag itself along, lay upon its side, bowels evacuated, bloody vomiting, and died in twenty-five minutes. Richet extracted two different bodies from the actinia poison: The crystalline *thallassin,* which had slight toxic action, but which in minimal doses caused pruritis, and *congestin,* an albuminous body which was more toxic than the original substance and caused the characteristic congestive conditions in the intestinal tract.

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[a] Pfeiffer, Hermann: Ueber die nekrotisirende Wirkung normaler Seren. Zeit. f. Hyg., Bd. 51, 1905, p. 183.

[b] Arthus: Injections répétées de sérum de cheval chez le lapin. Compt. rend., 1903.

[c] Richet: De l'anaphylaxie, ou sensibilité croissante des organismes à des doses successives de poison. Arch. di fisiol., 1904, p. 129. —— De l'action de la congestine sur les lapins et de ses effets anaphylactique. Compt. rend. Soc. biol., Jan. 21, 1905. —— De l'anaphylaxie après injections de congestine chez le chien. Compt. rend. Soc. biol., 1905.

Richet made further experiments with the congestin with relation to first and subsequent injections which gave him the same results as similar experiments with the actinia poison. The fatal dose for rabbits was 0.009 gm. per kilogram for the first injection; for the subsequent injection it was less than 0.0033. Richet designated this hypersensitiveness, caused by the first injection, by the term "anaphylactic" in contradistinction to prophylactic. This anaphylaxis requires for its development a certain time. Three to four days after the first injection there is no hypersensitiveness. It continues a very long time and is still evident after one year. He did not succeed in transmitting this hypersensitiveness by means of the serum of injected animals. He calls attention to similar phenomena in tuberculosis and tetanus.

Knorr,[a] as also Behring and Kitashima,[b] found that guinea pigs develop an increasing sensitiveness against tetanus toxin.

Serum and actinia poison are similar in that they are not substances capable of self-multiplication. On the other hand, there is this important difference between the two: The actinia poison causes after the first injection symptoms without a period of incubation, while the serum, even in the largest quantities, at the first injection almost without exception develops no immediate symptoms.

So far as serum is concerned, we may assert that the same is not toxic in itself, but that the toxic body is developed through the reaction between the organism and the antibodies.

Similar to serum are forms of animal albuminous substances as, for example, milk (Arthus) and spermatozoa (Wolff).

Entirely analogous to the actinia poison is the hypersensitiveness found in tuberculosis. The question in tuberculosis has been more closely studied in its relation to hypersensitiveness; another well-known fact is that in addition there also develops an immunity.

Tuberculin is an extract which lacks the power of self-multiplication, but is derived from an agent which has this power, and we would expect that infection with the tubercle bacillus would also carry the power of producing this hypersensitiveness the same as is produced by the extract from dead tubercle bacilli.

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[a] Knorr: Experimentelle Untersuchungen über die Grenzen der Heilungsmöglichkeit des Tetanus. v. 102, 18.

[b] Von Behring and Kitashima: Über Verminderung und Steigerung der ererbten Giftempfindlichkeit. Berl. klin. Woch., 1901, p. 157.

Here we come to speak of hypersensitiveness in infectious processes. Arloing, in 1888, stated as his opinion that the pathogenic organisms, above all the tubercle bacilli, excrete soluble substances that influence the organism in such a way that the animal at a later infection with the same bacilli dies more quickly.

Courmont[a] studied this question in relation to the tubercle bacillus. He found that bacilli obtained in pure culture from tuberculous pleurisy caused in rabbits general tuberculosis and kill guinea pigs without microscopic lesions. He injected animals with the filtered soluble products of young cultures of this bacillus.

Guinea pigs and rabbits never react to these injections. However, if the animals so prepared were immediately afterwards inoculated with pure cultures then they reacted, as did the untreated animals; but if the inoculation follows several days after the introduction of the soluble products, then the results were very different. Animals which twenty days previously had been injected with the filtrate, as a result of which they showed no reaction, were then inoculated with a tubercule from a guinea pig; then the guinea pigs died in fifteen and the rabbits in about twenty-three hours. The animals died sixteen times more quickly than the control animals. This predisposition seems to be at its height from four to twenty days after the first injection of the soluble extract. Courmont believes that the organism, as a result of the previous treatment, becomes a better culture medium for the infection. The gist of his explanation lies in the fact that the organism possesses for itself protective substances which are eliminated or neutralized by means of the soluble products of the first injection. This explanation is called, for short, the "absorption theory" (Ausschaltungs Theorie). Why several days are necessary for this action Courmont does not explain.

The school at Lyons developed this observation in relation to other micro-organisms, as the staphylococcus, streptococcus, bacillus pyocyaneus, etc. Rist made similar observations with the diphtheria bacillus.

Strauss and Gamaleia[b] report that animals, after repeated injections with dead tubercle bacilli, die acutely. They bear the first injection of large quantities without reaction.

Babes and Proca[c] confirmed this observation.

Against the "addition" theory of Koch, to which most of the German authorities adhere, and against the "absorption" theory of the

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[a] Courmont: Études sur les substances solubles prèdisposantes à l'action pathogène de leurs microbes producteurs. Rev. de méd., 1891. Centbl. f. Bakt., Bd. 13. p. 714.

[b] Strauss, L., and N. Gamaleia: Recherches expérimentales sur la tuberculose. Arch. de méd. expériment., III, 1899.

[c] Babes and Proca: Untersuchungen über die Wirkung der Tuberkelbacillen und über gegenwirkende Substanzen. Zeit. f. hyg., v. 23, p. 331.

Lyons school, Köhler and Westpfal[a] in 1891 announced a third theory to explain the action of tuberculin. The last-named authors believed that, through the union of tuberculin with the products of the tubercle bacilli in the tuberculous lesions, a third and new body results which causes the clinical manifestations of the tubercle reaction.

A similar explanation is used by Marmorek to account for the elevation of temperature. He believes that the tubercle bacilli, under the influence of tuberculin, secretes a pathogenic substance.

Bail, based upon the work of Detre-Deutsch, has taken up anew the work of Courmont and studied more precisely the hypersensibility in tuberculosis. To explain this hypersensibility in tuberculous guinea pigs he advances another theory, based upon the power to transmit the hypersensibility. For example, if we inject an animal, into the peritoneal cavity, at a certain stage of tuberculosis the animal dies rapidly. At the site of the injection an exudate is produced. If this exudate is added to tubercle bacilli and injected into normal animals it produces sudden death.[b]

Bail believes that the tubercle bacillus secretes a soluble substance in the infected organism which substance exerts a paralytic action upon the leucocytes. This substance he has called "aggressin." He believes that the exudate obtained by this reaction contains this aggressin and, when carried over, has the power of destroying the power of the leucocytes—that is, the protecting power of the new organism—and therefore the poisonous substance in the tubercle bacillus is able to develop its power quickly.

This explanation seems to be in line with Arloing and Courmont's.

In recent years Wolff[c] has also worked up the question of hypersensibility, proceeding upon the work of Pfeiffer upon the endotoxins. The endotoxin only possesses power when the bacterial capsules are dissolved by means of bacteriolysins that are freed by means of antibodies. As soon as the endotoxin is set free it exerts its power and, without a period of incubation, is a powerful poison.

The results of Pfeiffer and Wolff's observations upon the method of operation of the endotoxin are very closely related to von Pirquet and Schick's explanation of the production of the serum disease.

Wolff also experimented upon the question of hypersensibility caused by the reinjection of dissolved albuminous substances and his observations coincided with those of Arthus, Pirquet, and Schick. The endotoxic theory, in the sense of bacteriolysis, naturally can not be applied to albuminous substances in solution. We can only accept it in the sense that by means of the reaction between the antibodies and the antigen the poisonous substance is formed.

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[a] Kohler and Westphal: Eine neue Theorie zur Erklarung der Wirkung des Koch'sch en Heilmittels auf den tuberculosen Menschen. Deut. med. woch., 1891, p. 839.

[b] Bail: Ueber Empfindlichkeit bei tuberculosen Thieren. Weiner klin. woch., 1904. —— Der acute Tod von Meerschweinchen an Tuberculose. Loc. cit., 1905.

[c] Wolff, A.: Uber grundgesetze der immunität. Centblt. f. bakt., bd. 37, 1904, pp. 390, 566, and 684.

When it is necessary to conceive of bacteriolytic antibodies to produce solution in order to cause a reaction we must remember that the production requires a definite period of time. This led to the question of the cause of the period of incubation, the significance of which is important.

The long incubation period in tetanus, as has been demonstrated, may be explained by the fact that tetanus poison has a long distance to go along the nerve route before it comes into contact with the susceptible cells.

In the case of malaria the interval between the two paroxysms is explained by the fact that the symptoms of the disease are produced as a result of the particular phase in the developmental cycle of the parasite.

We have a similar conception of the incubation period of a number of diseases, for example, measles, smallpox, etc., in which we look upon the period of incubation as due to a breeding of the causative factor of the disease.

All of the above theories which account for the period of incubation of disease explain this phenomenon by referring it wholly and solely to the parasite and its metabolic products and do not take into account the factor of the infected organism. Pfeiffer and Wolff, however, in explaining the incubation period in infectious processes, take this factor into account: The rôle which the organism plays in the period of incubation in the infectious process becomes clearer from the studies upon the power of serum to produce a reaction.

Von Pirquet believes that, in his work on the hastening power of reaction in the case of vaccinia, the power which the organism plays in the period of incubation is very evident.

While the general reaction following the first vaccination at the very earliest occurs on the seventh day, it appears earlier upon revaccination. Also, the course of the reaction is changed in the sense of a decidedly shortening and weakening of the symptoms. The earlier the reaction appears the weaker does the whole process result.

How can we explain these phenomena? If we use the theories generally in vogue, that the cause of the infection must first multiply to certain numbers in order to be able to produce symptoms, we must believe that the cause of the infection develops more quickly and in larger numbers in the animal which has been previously treated than in the normal animal. We have already inferred that Arloing has suggested this possibility.

On the other hand, we must take into account that in tuberculosis and vaccinia, no matter how much of the causes of these infections we may introduce, it is not possible to shorten the period of incubation. Beclère, Chambon and Ménard[a] have shown that, in the vaccination of calves, the eruption does not appear sooner if the calf is very heavily treated with the vaccinal material.

We might attribute the phenomena to the virulence of the infecting organisms, but the same lymph causes the reaction to appear in animals primarily vaccinated in the normal period of incubation; whereas in those revaccinated there is a hastened reaction.

A related thought originated with Buttersack[b] in regard to infectious diseases who, in a study upon recovery and immunity, asserts that recovery is due to a retardation of the development of the cause of the infection at the height of the sickness. He believes further that the cells of the body, through use in the sense of Darwin's theory of evolution and adaptation, develop the power to combat more quickly the multiplication of a new infection.

Von Pirquet and Schick draw this distinction between Buttersack's theory and their own: Buttersack places this power of the organism to prevent a further development of the infection at the climax of the disease, while they believe the antibodies are formed at the very beginning. Their conception of antibodies does not coincide precisely with the generally accepted idea of antibodies, but they use this expression in a general sense to comprise the sum of all the specific reaction products produced by the organism which are able to react upon the antigens introduced. Buttersack's theory does not cover the explanation of the serum disease, as serum has no power of self-multiplication.

The sooner the reaction in the organism succeeds, the less time has the alien infection to develop. Therefore, its development is restricted earlier and, in consequence, the whole organism suffers less damage.

On the contrary, in the case of serum disease we receive the impression that it is unfavorable for the organism to react more quickly to the reagent. For the quicker the antibodies unite with the antigens the more severe will the disease be.

This contradiction may perhaps be cleared up when we remember that the subcutaneous injection of an agent incapable of self-multiplication seldom occurs in nature (insect bites, snake poison) and that this to a certain extent represents an artificially produced form of disease. The natural way for disease to be produced is, in the great majority of cases, by the introduction of minute quantities of an irritant capable of self-multiplication.

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[a] Beclère, Chambon, and Ménard: Étude expérimentale des accidents postsérotherapiques. Ann. de l'Inst. Pasteur, 1896. —— Études sur l'immunité vaccinale. Ann. de l'Inst. Pasteur, 1896 and 1899.

[b] Buttersack: Immunität und Heilung im Lichte der Physiologie und Biologie. Virchow's Arch., Bd. 142, p. 248.

The hastening power of the reaction must be regarded as an advantage that the organism has won for itself through the occurrence of the first disease.

In applying this view to disease von Pirquet and Schick find that an organism in the stage of the free antibodies is an expression of the immediate power of reaction or hypersensitiveness. This stage has only a limited duration. The free antibodies disappear; however, the individual remains immune. The fact of this immunity consists, however, no longer in the power of immediate reaction against the cause of the infection, but in a hastened power of reproduction of antibodies. An organism has the power to combat a newly introduced infection and localize it, as is well shown in the case of vaccination. Von Pirquet and Schick believe that this property does not depend so much upon free antibodies in the fluids as upon a property acquired by the cells through the first disease, in which we see an expression of cellular immunity.

In Wassermann's[a] observation that the adaptation of the tissues to the existence of microorganisms which were naturally pathogenic without any apparent reaction and production of antibodies we see an instance of immunity through insusceptibility.

A series of diseases—for example, smallpox, measles, varicella, röthlen—cause after one attack the individual to remain more or less protected throughout the rest of his life. They have the common clinical characteristic that, after a long and definite period of incubation, the disease runs a course in a definite and comparatively short time. If the organism does not die as a result of the disease, then it has won a complete victory over the cause of infection; the same is no longer able to produce harm in such an organism.

According to von Pirquet and Schick the fact that the clinical immunity against this group of diseases, for which we may take vaccinia as a type, does not consist of an acquired nonsusceptibility against the cause of the infection, but the power of an accelerated reaction. They believe they have shown from their work that they have reawakened interest in the serum disease and that this syndrome is not only of interest clinically but, from the standpoint of general pathology, is of the greatest importance.

Our explanation of the cause of sudden death following the second injection of horse serum does not differ essentially from the theory (which has just come to our notice as our manuscript is being edited for press) published by von Pirquet and Schick, relative to the reaction of the serum disease in man, although these similar deductions were reached independently and from different premises.

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[a] Wassermann, A.: Uber natürliche und künstliche Immunität. Zeit. f. Hyg., bd. 37, p. 173. —— Wesen der Infection, in Kolle und Wassermann. Handb. der pathogen. Mikroorganismen, 1903.

We believe that the substance which sensitizes the animal is identical with that which later poisons it, absurd as that may seem at first glance. However, the first substance must cause a reaction in the organism resulting in the production of "antibodies" and it is these antibodies combining with a substance in the horse serum that produce the toxic action. We have found that small quantities of serum produce, after a definite period of incubation, a condition of anaphylaxis. Large quantities probably produce a lesser grade of hypersusceptibility. Multiple or repeated injections produce immunity. We therefore possess in horse serum a substance capable of causing both anaphylaxis and prophylaxis.

SUMMARY AND CONCLUSIONS.

Normal horse serum, when injected into the peritoneal cavity of a normal guinea pig, produces no symptoms. When injected subcutaneously there may result at most a slight local reaction consisting of swelling and edema, which gradually disappears.

Antitoxic horse serum is equally harmless for normal guinea pigs.

Horse serum is, however, poisonous to a guinea pig which has previously been injected with horse serum. The "period of incubation" or time necessary to elapse between the first and second injection is about ten days. Under these circumstances, horse serum is poisonous whether injected subcutaneously or into the peritoneal cavity.

The first injection of horse serum renders the guinea pig susceptible.

The symptoms caused by the injection of horse serum into a susceptible guinea pig are respiratory embarrassment, paralysis, and convulsions, followed by death. The symptoms come on usually within ten minutes after the injection, and when death results it usually occurs within one hour, frequently in less than thirty minutes, and sometimes within a few minutes.

The poisonous principle in horse serum appears to act upon the respiratory centers. The heart continues to beat long after respiration ceases.

The toxic action of horse serum bears no relation to diphtheria. The poison is not *toxone.* Guinea pigs can not be rendered susceptible by previous infections with the *B. diphtheriæ* or by previous injections with diphtheria toxine.

It seems from our work, however, that guinea pigs first injected with a mixture of diphtheria toxine plus horse serum are more sensitive to subsequent injections of horse serum than are guinea pigs sensitized with a first injection of horse serum alone.

DIPHTHERIA ANTITOXIN PLAYS NO PART IN THIS POISONOUS ACTION AND IN ITSELF IS HARMLESS.

As soon as we realized that the toxic principle in horse serum exerts its action in quantities so minute as to place it almost in the category of the ferments and, further, when we concluded from our work that this toxic principle is doubtless one of those highly organized and complex proteid substances belonging to the "haptin group" in the sense used by Ehrlich, we recognized how futile it would be with present methods to attempt to isolate this substance.

Nevertheless we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eliminating or neutralizing this toxic principle in horse serum is at once evident.

It is probable that when the strange proteid is introduced into the guinea pig it causes a reaction resulting in a production of "antibodies," so that when a second injection of horse serum is given there is probably either a union or a reaction between the antibodies and a substance in the horse serum which produces the poisonous effect.

This poisonous principle is quantitatively specific; that is, guinea pigs treated with horse serum are rendered somewhat susceptible to the subsequent injection of the serum of another animal. Guinea pigs treated with the serum of another animal are slightly sensitive to the toxic action of horse serum.

Guinea pigs treated with the serums of various animals and subsequently injected, are much more susceptible to homologous serums than to heterologous serums.

This poisonous action has no relation to hemolysis. Our work proves that blood serum may contain an acute poison entirely independent of any hemolytic action. Normal horse serum has no lytic power upon the red corpuscles of the normal guinea pig.

This poisonous action has no relation to the specific albuminous precipitins.

The poisonous principle in horse serum is not affected by a temperature of 60° C. for 6 hours, but it is destroyed at 100° C. for 15 minutes.

The poisonous principle is filterable through porcelain, is not injured by drying, and can not be separated by precipitation with ammonium sulphate and subsequent dialysis.

The following chemical substances do not oxidize, neutralize, or precipitate the poisonous principle in horse serum: Butyric acid, permanganate of potash, citrate of soda, alcohol, succinic peroxide acid (alphozone), hydrogen dioxide, and ammonium sulphate. The presence of chloroform or trikresol (0.4 per cent) does not interfere with this poisonous action.

Serums eight years old are as toxic as those freshly separated.

Exposure to X-rays does not affect the poisonous action of horse serum.

It requires about 10 days after the first injection of horse serum for a guinea pig to show susceptibility to a second injection. A guinea pig remains susceptible a very long time, at least 160 days.

As small a quantity as $\frac{1}{1,000,000}$ c. c. of horse serum was sufficient in one instance to render a guinea pig susceptible. Quantities varying from $\frac{1}{250}$ to $\frac{1}{1000}$ c. c. almost invariably render guinea pigs highly susceptible when given in the toxine-antitoxine mixture.

One-tenth c. c. of horse serum injected into the peritoneal cavity of a susceptible guinea pig is sufficient to cause death. The same quantity inoculated subcutaneously may cause serious symptoms.

There is some evidence to show that the sensitizing substance in horse serum is the same as the poisonous substance. The sensitizing substance is not affected by precipitation with ammonium sulphate and dialysis.

Guinea pigs may be sensitized with horse serum that has been dried and redissolved.

The sensitizing substance is not affected by a temperature of 60° C. for 6 hours.

It is probable that small quantities of horse serum render a guinea pig more susceptible than do large quantities. If this be true, it is due, perhaps, to the fact that large quantities, owing to slow absorption or prolonged reaction, partly immunize the guinea pig at the same time that it is being sensitized.

The sensitizing substance apparently is not free in the blood serum of guinea pigs.

An active immunity against this toxic principle may readily be established by repeated injections of horse serum, at short intervals, into a guinea pig. Although guinea pigs may be immunized actively in this manner we have not yet succeeded in transferring this immunity in the blood serum or body juices to another guinea pig. It therefore appears that the immune bodies, if such exist against the toxic action of horse serum, are not free in the blood and body juices contrary to the case in diphtheria.

Guinea pigs may be sensitized to the toxic action of horse serum by feeding them with horse serum or horse meat.

The fact that guinea pigs may be rendered susceptible by the feeding of strange proteid matter opens an interesting question as to whether sensitive guinea pigs may also be poisoned by feeding with the same serum given after a proper interval of time. If man can be sensitized in a similar way by the eating of certain proteid substances may not this throw light upon those interesting and obscure cases in which the eating of fish, sea food, and other articles of diet habitually cause sudden and sometimes serious symptoms?

The susceptibility to the toxic action of horse serum is transmitted hereditarily from the mother guinea pig to her young.

These results upon the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea pigs may throw light upon the well-known hereditary tendency to tuberculosis in children born of a tuberculous parent. There are certain analogies between the action of tuberculosis and horse serum. Both may produce a hypersusceptibility and also a certain degree of immunity. Now that we have proved that this hypersusceptibility or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea pigs, may it not throw light upon the fact that tuberculosis "runs in families?"

Demonstrations of the hereditary transmission of acquired characters are comparatively rare in biology. While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young, yet, as far as we know, this is the first recorded instance in which hypersensitiveness, or anaphylaxis, has been experimentally shown to be transmitted from a mother to her young.

Other albuminous substances, such as skimmed milk, peptone, hemoglobin, egg albumin, and vegetable proteids possess no poisonous action upon guinea pigs sensitized with horse serum. Whether guinea pigs are rendered susceptible to a subsequent injection with the same albuminous matter with which they have been sensitized will be reported in a later paper.

We believe that the substance which sensitizes the animal is identical with that which later poisons it. However, the substance must first cause a reaction in the organism resulting in a production of antibodies. We have found that small quantities of horse serum produce, after a definite period of incubation, a condition of anaphylaxis; multiple or repeated injections produce immunity. We therefore possess in horse serum a substance capable of causing both anaphylaxis and prophylaxis.

It may be that man can not be sensitized in the same way that we have shown to be the case with guinea pigs. Children have, in a number of instances, been injected with antidiphtheric horse serum at short and long intervals without, so far as we are aware, causing death. Certain serums, for example, the antitubercle serum of Maragliano and the antirheumatic serum of Menzer, are habitually used by giving injections at intervals of days or weeks. In all such cases of frequent and repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to our work. Von Pirquet and Schick have shown that a second injection of horse serum into children causes an "immediate" or an "accelerated" reaction. Both the immediate and the accelerated reaction in children are characterized by symptoms of "the serum disease."

We might conclude that children may not be sensitized to the toxic action of horse serum by eating horse meat, for horse meat is a favorite article of diet in certain European countries and there is nothing upon record to show that the injection of horse serum in those countries is fraught with more danger than where this diet is not used. It should, however, be borne in mind that our work has shown that guinea pigs may be sensitized with exceedingly minute quantities of a strange proteid, and that repeated injections cause an immunity, and it does not seem impossible that the same action may be true of food.

Man reacts to the first injection of horse serum after a period of eight to thirteen days; guinea pigs show no reaction as a result of the first injection; both man and guinea pigs react to a second injection. The reactions in man and the guinea pig differ, however, both in severity and in kind. The relation, therefore, that our observations upon the guinea pig may have in their application to man must await further study.

The fact that other animals beside man and the guinea pig react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action.

We believe that our results make it probable that man may be rendered sensitive to the injection of a strange proteid, as is the case with the guinea pig and other animals, and that this explanation must be considered as well as the *status lymphaticus*, which has heretofore been assigned as the cause of sudden death following the injection of horse serum.

[Post Scriptum.—After the galley proof of this article had left our hands an article by R. Otto entitled "Das Theobald Smithsche Phänomen der Serum-Ueberempfindlichkeit," reprinted from Leuthold-Gedenkschrift, band 1, first came to our notice. His paper deals with some of the problems we have studied and his results are in harmony with many of our conclusions.]

O

(Continued from second page of cover.)

No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

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No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

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Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 30.

M. J. ROSENAU, Director.

August, 1906.

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I.—MATERNAL TRANSMISSION OF IMMUNITY TO DIPHTHERIA TOXINE.

II.—MATERNAL TRANSMISSION OF IMMUNITY TO DIPHTHERIA TOXINE AND HYPERSUSCEPTIBILITY TO HORSE SERUM IN THE SAME ANIMAL.

BY

JOHN F. ANDERSON.

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The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

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No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

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No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

(Continued on third page of cover.)

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1906.

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I.

MATERNAL TRANSMISSION OF IMMUNITY TO DIPHTHERIA TOXINE.

By John F. Anderson,

Passed Assistant Surgeon, Assistant Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

When the Hygienic Laboratory undertook the work of preparing a standard unit for the measurement of antidiphtheric serum, one of the first necessities was a constant and reliable supply of guinea pigs weighing from 250 to 280 grams. It was estimated that 500 females in the breeding pens would insure a sufficient number for this, as well as for the other work of the laboratory. It occurred to me that perhaps we might use as breeders the pigs that had been used for the testing of antitoxin or determining the strength of diphtheria toxine and had fully recovered. Before placing such pigs in the breeding pens I thought it would be well first to test the young of a number of such used and recovered pigs to see if they were resistant to the action of diphtheria toxine. For this purpose 21 pigs were placed aside and their young tested either by the giving of a MLD or L+ dose, control pigs being used in every case from the stock pigs whose mothers had previously had no treatment. All of the animals were under exactly the same conditions as regards food, temperature, air, light, etc., both before and after testing.

The determination of this question is of a threefold importance:

First. We know from work by Rosenau and Anderson[a] that there is a hypersusceptibility to the injection of horse serum, normal or antitoxic, transmitted by a mother pig which has had a toxine-antitoxin mixture or serum alone to her young. It is, therefore, manifest that young pigs from such mothers should not be injected with serum for the purpose of determining the absence of bacterial contamination. In this test it is usual to inject several cubic centimeters of the serum

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[a] Rosenau, M. J., and John F. Anderson: A study of the cause of sudden death following the injection of horse serum. Hygienic Laboratory Bulletin No. 29, U. S. Public Health and Marine-Hospital Service. Washington, 1906. 95 p. 8°.

Manuscript submitted for publication June 26, 1906.

under examination, and in the work referred to 1 c. c. was sufficient to cause the death of pigs with a hypersusceptibility transmitted from the mother.

Second. If treated and recovered pigs are used as breeders and their young prove more resistant to diphtheria toxine, then when tests of serum are made on such resistant pigs we would evidently obtain misleading results.

Third. If these young pigs are of average susceptibility the recovered pigs could be used as breeders, resulting in quite a saving in animals bought for breeding purposes.

The use of such used animals as breeders was the custom in two antitoxin laboratories that I personally know of, and I suggested it as one explanation of irregularities they encountered in the testing of antitoxin. Both eliminated these used animals from their breeding pens with a decided decrease in the number of irregular results.

No pigs have been bought for use in the Hygienic Laboratory since May 31, 1905, the number raised being ample. We obtained pigs from three sources for breeding purposes, by far the larger number being presented to the laboratory by the Bureau of Animal Industry, none of which, I am sure, for many generations back had any taint of diphtheria toxine. As to the other two sources of supply I can not be so sure; but all of those received at the laboratory were under 300 grams in weight and consequently would not have been used for work with diphtheria toxine. Since May, 1905, we have used 3,439 pigs for the testing of diphtheria toxine or antitoxin. During this time a careful search of the records shows a very small number of pigs which died much earlier than was to be expected with the dose given, and some of these presented pneumonia at the autopsy. Only three have failed to die acutely when used on the L+ dose, thus showing very plainly that marked resistance to diphtheria toxine is unusual in the pigs bred at the Hygienic Laboratory.

Theobald Smith,[a] in giving the results of tests of the young of three female pigs in which there was an immunity transmitted from the mother, says that this may be due to (1) the influence of any preliminary treatment, (2) the influence of the male, (3) the influence of selection in breeding. He considers his data sufficient to rule out the first, but states that the mother of his most resistant pigs (No. 2944) had previously been treated with a toxine-antitoxin mixture and about four or five months afterwards put into the breeding pens. I think, in view of my results, that this treatment fully accounts for the immunity of her young.

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[a] Smith, Theobald: Degrees of susceptibility to diphtheria toxin among guinea pigs; transmission from parents to offspring. Journ. med. research, vol. 13, n. s. vol. 8, Feb., 1905, p. 341-348.

In regard to his third question I think that the results obtained at the Hygienic Laboratory with the pigs raised here show that in our guinea pigs, at least, insusceptibility to diphtheria toxine is very rare. I am inclined to think that the influence of artificial selection in breeding is of slight importance if previous treatment of the breeders with the diphtheria toxine-antitoxin mixture can be excluded for several generations back. I can conceive that by the selection of breeders who have shown a special resistance to diphtheria toxine a very resistant race of pigs may be bred.

It is not improbable that dealers and breeders of guinea pigs may buy at times used guinea pigs from persons engaged in work with diphtheria antitoxin and thus introduce unknowingly into their stock toxine-resisting pigs.

The first experiments to immunize animals during pregnancy against pathogenic bacteria were positive.[a] Chauveau found that the young of sheep so immunized were immune to anthrax, and like results were arrived at by Arloing, Cornevin, and Thomas in symptomatic anthrax.

Ehrlich was the first to study the problem methodically, and his results have been confirmed and stand. He used mice that had acquired a high degree of antitoxic immunity through systematic feeding of ricin, abrin, and robin. He showed that the male (sperm) element was incapable of transmitting the immunity.

Ehrlich used female animals that were immune before becoming pregnant. By using animals that were being immunized during pregnancy his results were interpreted as negative, inasmuch as positive results could not be referred to an active intra-uterine immunization of the fetus. In all of these cases his results were positive in that about four weeks after birth a definite immunity was demonstrable. About one and a half months after birth there still remained some undoubted immunity, but in the course of three months all traces had disappeared.

This short immunity in the progeny of mothers was considered as passive immunity and rested on the transmission of antibodies from the mother. Against inheritance in the strict sense there was the fact that there was absolutely none present in the grandchildren of immune mothers.

Ehrlich, therefore, concludes that neither sperm nor germ transmits the immunity and so no inheritance in the strict sense takes place.

Ehrlich, by his "mother exchange" experiment, showed that the antitoxins furnished in intra-uterine life did not long (21 days) remain in the young organism. He showed positively that the milk was the vehicle by which the suckling organism received its antitoxin and with the length of the suckling furnishes it with an increasing immunity. The long persistence of the immunity depended upon a transfer of antibodies through the milk. By the immunization of a nursing mother mouse (after birth of the litter) Ehrlich was able to transmit immunity to swine-plague to the nursing young.

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[a] The above summary of a part of the literature upon the influence of heridity in immunity is largely taken from the article by Morgenroth in Handbuch der pathogenen Mikroorganismen, 4 bd., p. 784. Those interested will find this an excellent discussion of the subject with bibliography.

Ehrlich and Hübener showed that Tizzoni and Centani had erred when they appeared to show that in rabbits the progeny of a male immune to hydrophobia and a female immune to tetanus was immune to hydrophobia. In experiments with tetanus-immune guinea pigs and mice they obtained results in entire harmony with the previous results of Ehrlich; and, opposed to Tizzoni's reports, established that in tetanus also no transmission occurs through a male, but that only through the mother does transmission of immunity take place which, by the end of the second and positively by the end of the third month, disappears.

Naillard came to the same conclusions from experiments on guinea pigs and rabbits.

The results of Wernicke on diphtheria-immune guinea pigs were also the same. "In diphtheria the immunity is not transmitted by the father, but the mother only." This immunity can not be detected in the grandchild, but remains longer in the first generation (the children), for in three months a considerable immunity is still present.

Transmission of immunity through the milk also occurs in guinea pigs.

Klemperer found in eggs of tetanus-immune hens tetanus antitoxin in the yolk, not in the white.

Kitt injected hens with eggs derived from chicken-cholera-immune hens and obtained immunity.

Sclavo immunized hens against diphtheria by injection of weakened cultures, and found that the whites of the eggs protected guinea pigs against lethal doses of diphtheria bacilli.

Tables Nos. 1 and 2 show the results of tests of the young of female guinea pigs that had recovered from the effects of an injection of toxine alone or of the toxine-antitoxin mixture. These animals were put aside for breeding after having been under observation 35 days after injection, which is the time all animals used for antitoxin work in the laboratory are kept under observation.

Table No. 1 shows the results of the tests of the young pigs against an L+ dose, and Table No. 2 against an MLD.

Table No. 1.—*Showing resistance to the L+ dose in guinea pigs born of a mother treated with a single injection of toxine or toxine-antitoxin mixture.*

Mothers.							Young pigs.						Controls.	
	Treatment.				Result.			Dose.		Result.			Result.	
	Toxine.		Serum.								Recovery.			
No.	No.	Amount.	No.	Amount.	Reaction.	Paralysis.	No.	Toxine.	Serum.	Death.	Reaction.	Paralysis.	Death.	Recovery.
608	29*a*	0.002	0	0	Ulcer.	—	608A	*0.22	1 *iu*	3-22	....	....	2-19	....
1424	9	.12	Ehr.	1 *iu*	—	—	1424A	.22	1 *iu*	8-3	....	....	5-7	....
							1424B	.22	1 *iu*	7-2	....	....	5-0	....
1992	9	.23	A4	1 *iu*+	—	—	1992A	.22	1 *iu*	......	—	—	2-6	....
1524	7	.21	B14	1 *iu*+	—	+	1524C	.22	1 *iu*	......	Ulcer.	—	2-1	....
1909	5	.133	140	$\frac{1}{400}$	—	—	1909A	.22	1 *iu*	2-15	....	....	2-15	....
1773	17A	.006	0	0	Ulcer.	—	1773A	.22	1 *iu*	2-17	....	....	3-7	....
1909	5	.133	140	$\frac{1}{400}$	—	—	1909B	.22	1 *iu*	3-16	....	....	2-22	....
608	29*a*	.002	0	0	Ulcer.	—	608E	.22	1 *iu*	3-8	....	....	2-12	....
1907	5	.133	137	$\frac{1}{250}$	—	—	1907A	.22	1 *iu*	3-3	....	....	3-3	....
1946	15A	.3	B17	1 *iu*	—	—	1946C	.22	1 *iu*	......	Induration.	—	3-3	....
							1946A	.22	1 *iu*	......	Induration.	....	2-17	....
							1946B	.22	1 *iu*	......	Ulcer.	—	3-0	....
1766	17A	.005	0	0	Ulcer.	—	1766A	.22	1 *iu*	3-0	....	....	3-0	....
							1766B	.22	1 *iu*	2-19	....	....	3-0	....
1902	5	.133	181	$\frac{1}{600}$	—	—	1902A	.22	1 *iu*	......	Ulcer.	+	2-12	....
							1902B	.22	1 *iu*	......	Ulcer.	+	2-12	....
1905	5	.133	137	$\frac{1}{200}$	—	—	1905A	.22	1 *iu*	......	Ulcer.	+	3-0	....
2264	19	.007	0	0	Ulcer.	+	2264C	.22	1 *iu*	2-14	....	....	2-12	....
3827	7	.22	201	$\frac{1}{350}$	—	—	3827C	.22	1 *iu*	......	Ulcer.	-	3-2	....
608	29*a*	.002	0	0	Ulcer.	—	608B	†.48	1 *iu*	2-23	....	....	2-3	....
1524	7	.21	B14	1 *iu*+	—	+	1524A	†.49	1 *iu*	......	Ulcer.	+	2-21	....
							1524B	.49	1 *iu*	7-5	....	....	2-21	....
1277	5	.130	IX-7	1 *cc*	—	--	1277B	†.50	1 *iu*	3-20	....	....	3-6	....

* No. 7.

† No. 15.

A study of this table shows that of 14 mothers, 7, or 50 per cent, transmitted an immunity to their young sufficient to withstand the effects of an L+ dose; all living except Nos. 1424A, 1424B, and 1524B, which lived 3, 2, and 4 days, respectively, longer than their controls. Of a total number of 24 young pigs shown in this table, 13, or 54.16 per cent, were resistant to an L+ dose.

Of the mothers that received the toxine-antitoxin mixture, 7, or 70 per cent, transmitted an immunity to their young.

The fact that no mother which received a dose of toxine alone transmitted an immunity to her young is very plainly shown.

Table No. 2.—*Showing resistance to the MLD of guinea pigs born of a mother treated with a single injection of toxine or a toxine-antitoxin mixture.*

Mothers.							Young pigs.						Controls.	
	Treatment.				Result.			Dose.		Result.			Result.	
	Toxine.		Serum.								Recovery.			
No.	No.	Amount.	No.	Amount.	Reaction.	Paralysis.	No.	Toxine	Serum	Death	Reaction.	Paralysis.	Death.	Recovery.
608	29a	0.002	0	0	Ulcer.	–	608F	*0.005	0	2-5	....	....	2-18	....
1278	5	.130	IX-17	$\frac{1}{100}$	–	–	1278C	*.005	0	.....	Ulcer.	+	4-1	....
1907	5	.133	137	$\frac{1}{200}$	–	–	1907B	*.005	0	.....	Ulcer.	–	.....	...
1277	5	.130	IX-7	1 cc	–	–	1277A	* 006	0	3-12	....	....	2-13	....
1440	31B	.006	–	–	Indurated.	–	1440B	*.006	0	2-10	....	...	2-5	....
1445	34A	.006	0	0	Ulcer	–	1445A	*.006	0	2-8	....	....	2-21	....
608	29a	.002	0	0	Ulcer		608D	† 01	0	4-0	......	...	3-7	....
1278	5	.130	IX-17	$\frac{1}{100}$	–		1278A	† 01	0	.....	Ulcer.	–	3-19	...
1440	31B	006		–	Indurated	–	1446A	† 01	0	2-21	....	...	5-3	....
1773	17A	.006	–	–	Ulcer	–	1773B	† 01	0	4-25	....	...	4-9	....
1445	34A	006	0	0	Ulcer	–	1445B	† 02	0	2-7	....	..	2-5	....
1277	5	130	IX-17	1 cc	–	–	1277C	† 02	0	2-12	....	...	2-12	....
1278	5	130	IX-17	$\frac{1}{100}$	–	-	1278B	† 02	0	.....	Ulcer	–	2-12	....
1426	9	.16	Ehr	1 m	–	–	1426A	‡.006	0	4-0	....	...	3-3	....
1427	9	.18	Ehr.	1 m	–	-	1427B	‡ 006	0	.....	Ulcer	–	2-17	....
1497	5	.130	B14	1 m+	–	+	1497A	‡ 006	0	....	Ulcer	–	2-22	....
							1497B	‡ 006	0	....	Ulcer	–	3-8	....
1905	5	.133	137	$\frac{1}{200}$	–	–	1905B	‡ 006	0	......	Ulcer	+	3-6	....
							1905C	‡ 006	0	......	Ulcer	+	3-6	....
4495	7	.19	B27	1 m	+	–	4495A	‡.007	0	......	–	....	2-2	....
							4495C	‡ 007	0	......	-	....	1-12	....

*No 9 †No 15 ‡No 7.

An examination of this table shows that of 12 mothers 6, or 50 per cent, transmitted an immunity to their young.

Of a total number of 21 young pigs treated 11, or 52 per cent, were resistant to a fatal dose for control pigs.

Of the mothers that received the toxine-antitoxin mixture 75 per cent transmitted an immunity to their young.

No mother that had received an injection of toxine alone transmitted this immunity.

It will be noticed in both tables that where an immunity is transmitted to the young all of the litter are immune to about the same degree.

The question will naturally be asked, Why is this immunity not transmitted by all of the mothers treated with the toxine-antitoxin mixture? I can not answer this question positively, but suggest that it may be due to the fact that some of the pigs produce more antitoxin than others. It is a well-known fact among producers of diphtheria antitoxin that some horses can never be made to produce a serum of even 250 units, which is considered a rather low potency serum. Such may be the case with guinea pigs.

The following data, kindly furnished me by Dr. J. J. Kinyoun, director of the biologic laboratories of H. K. Mulford & Co., is of interest in this connection. It will be seen by reference to the table (No. 3) that some horses, in spite of prolonged treatment, never produce any antitoxin.

TABLE No. 3.—*Table showing that some horses, in spite of prolonged treatment, fail to produce any antitoxin, or only small amounts.*

No.	Duration of treatment.	Number MLD for 250 gm. G. P.	Number of trials.	Highest value.	Result.
				Units.	
1	14 weeks	1,280,000	4	0	Died.
2	18 weeks	1,110,000	5	50	Do.
3	19 weeks	1,010,000	4	100	Do.
4	20 weeks	2,080,000	5	100	Do.
5	.....do	1,800,000	3	100	Do.
6	22 weeks	1,520,000	5	0	Returned.
7	.....do	2.390,000	10	100	Do.
8	24 weeks	1,980,000	6	0	Died.
9	25 weeks	2,810,000	12	100	Do.
10	.....do	2,590,000	5	0	Transferred.
11	.....do	3,990,000	8	0	Do.
12	26 weeks	5,710,000	12	100	Died.
13	28 weeks	3,520,000	10	100	Returned.
14	30 weeks	3,770,000	6	100	Died.
15	32 weeks	4,600,000	12	0	Transferred.
16	36 weeks	5.530,000	11	50	Died.
17	.....do	4,000,000	15	100	Do.
18	37 weeks	4,500,000	14	100	Returned.
19	39 weeks	6,030,000	9	100	Died.
20	47 weeks	19.500,000	23	150	Transferred.
21	48 weeks	10.560,000	20	150	Do.
22	.....do	9.090,000	17	100	Returned.

Attention is called especially to horses Nos. 1, 6, 8, 10, 11, and 15, which never produced serum with antitoxic properties.

THE EFFECT OF REPEATED INJECTIONS IN THE MOTHER ON HER YOUNG.

At the suggestion of Doctor Rosenau a number of female guinea pigs were given repeated injections either of antitoxin alone or of one unit of antitoxin plus slightly more than the L^0 dose of toxine in order to study the effects of repeated injections in the mother on her young. An untreated male was placed in the pen with them. The young were allowed to remain with their mother until the day before being tested. They were tested in most cases against an L+ dose, though in some cases a larger dose, either one and a third or one and a fourth times the L+ dose, was given.

Pigs Nos. 4E, 4F, 8G, and 8H received eight and one-third times the MLD.

GUINEA PIG NO. 4.

May 25, 1905. Given 0.15 c. c. toxin No. 7 (L+ .21) +1 immunity unit Ehrlich 15. IV .05.
May 29. Same treatment.
June 9. 0.18 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. IV .05.
June 16. 0.19 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. IV .05.
June 26. 0.19 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. IV .05.
July 12. 0.19 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. IV .05.

Litter No. 1.

July 28, 1905. 4A and 4B born.
August 25. 4A given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. VIII .05. Moderate reaction at site on third day. Recovered. Control died in 3 days and 3 hours.
August 25. 4B given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. VIII .05. Died in 6 days and 3 hours. Control died in 3 days and 3 hours.

Litter No. 2.

November 7, 1905. 4C and 4D born.
January 6, 1906. 4C given 0.22 c. c. toxine No. 7+1 immunity unit B27. No reaction. Control died in 3 days and 7 hours.
January 24. 4D given 0.293 c. c. toxine No. 7 (=1⅓ times the L+ dose). Induration followed by small ulcer. Recovered. Control died in 1 day and 15 hours.

Litter No. 3.

May 21, 1906. 4E and 4F born.
June 22. Both given 0.05 c. c. toxine No. 7 (MLD=0.006 c. c.). Slight induration third day. Controls died in 1 day and 4 hours and 22 hours, respectively

GUINEA PIG NO. 5.

Same treament as No. 4.

Litter No. 1.

July 27, 1905. 5A and 5B born.
September 9. Both given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. IX .05. Slight induration at site in both pigs. Recovered. Controls died in 3 days and 12 hours.

Litter No. 2.

October 27, 1905. 5C and 5D born.

January 6, 1906. Both given 0.22 c. c. toxine No. 7 + 1 immunity unit B27. No reaction in either pig. Controls died in 3 days and 7 hours.

Litter No. 3.

February 3, 1906. 5E born.

March 17. Given 0.293 c. c. toxine No. 7 (1⅓ times the L + dose) + 1 immunity unit B28. Very slight reaction fourth day. Recovered. Control died in 1 day and 18 hours.

Litter No. 4.

April 12, 1906. 5F and 5G born.

June 5. Both given 0.275 c. c. toxine No. 7 (1¼ times the L + dose) + 1 immunity unit Ehrlich 15. VI .06. Both showed induration followed by ulcer at site. Control died in 1 day and 21 hours.

GUINEA PIG NO. 6.

Same treatment as No. 4.

Litter No. 1.

September 7, 1905. 6A and 6B born.

October 20. Both given 0.22 c. c. toxine No. 7 + 1 immunity unit B25. No reaction. Controls died in 3 days and 2 hours.

Litter No. 2.

November 8, 1905. 6C, 6D, 6E, and 6F born; 6C, 6E, and 6F used for other work.

January 24, 1906. 6D given 0.293 c. c. toxine No. 7 (1⅓ times the L + dose) + 1 immunity unit B27. Small ulcer. Recovered. Control died in 1 day and 20 hours.

Litter No. 3.

February 17, 1906. 6G born.

March 17. Given 0.293 c. c. toxine No. 7 (1⅓ times the L + dose). Very slight reaction. Control died in 1 day and 18 hours.

Litter No. 4.

April 26, 1906. 6H and 6I born.

June 5. Both given 0.275 c. c. toxine No. 7 (1¼ times the L + dose). No reaction in either pig. Control died in 1 day and 21 hours.

GUINEA PIG NO. 7.

May 25, 1905. Given 250 units antitoxin.

June 9. Given 600 units antitoxin.

June 16. Given 900 units antitoxin.

June 26. Given 1,000 units antitoxin.

July 12. Given 1,500 units antitoxin.

Litter No. 1.

July 26, 1905. 7A born.

August 16. Given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15. VIII .05. Small ulcer. Recovered. Control died in 3 days and 7 hours.

August 24. The mother pig was killed, blood drawn, allowed to clot, and serum tested to 12, 25, and 50 units. All the pigs died in about 24 hours, showing that her serum contained considerably less than 12 units per cubic centimeter.

GUINEA PIG NO. 8.

Same treatment as No. 7.

Litter No. 1.

July 29, 1905. 8A, 8B, and 8C born. 8B and 8C died.

September 15. 8A given 0.22 c. c. toxine No. 7 — 1 immunity unit Ehrlich 15. VIII .05. Died in 7 days and 7 hours. Control died in 4 days.

Litter No. 2.

March 15, 1906. 8D, 8E, and 8F born.

April 27. 8D given 0.22 c. c. toxine No. 7 — 1 immunity unit Ehrlich 15. VIII .06. No reaction. Control died in 4 days and 8 hours.

April 27. 8E and 8F given same injection as 8D. Both died on the third day. Controls died on the third day.

Litter No. 3.

May 20, 1906. 8G, 8H, and 8I born.

June 22. 8G and 8H given 0.05 toxine No. 7 (MLD = 0.006 c. c.). 8G died in 1 day and 4 hours. Control died in 22 hours. 8H showed slight induration on third day. Recovered. Control died in 1 day and 4 hours.

A study of the above results shows that the young of all the mothers that were treated by repeated injections of the toxine-antitoxin mixture had a marked resistance to an L+ dose; all survived except one, 4B, which died, but lived 3 days longer than the control. Four (4D, 5E, 6D, and 6G) withstood one and a third times the L+ dose; this dose uniformly caused the death of the controls in less than 2 days. Four (5F, 5G, 6H, and 6I) resisted one and a fourth times the L+ dose.

Of four pigs tested against eight and a third times the MLD three recovered (4E, 4F, and 8H); one (8G) showed the average susceptibility to the toxine.

The members of the fourth litter are as resistant as those of the first. The immunity seems to be greater in those pigs born of mothers treated with the toxine-antitoxin mixture than in those treated with the antitoxin alone. Pigs Nos. 4, 5, and 6 received a total of only 6 units, given in doses of 1 unit plus an amount of toxine slightly greater than the L^0 dose, while pigs 7 and 8 received 4,250 units. Of seven young of these last two mothers, three recovered, one lived 3 days longer than the control, and three showed no resistance.

Moreover, it would seem that the immunity produced by the injection of large amounts of antitoxin alone is not as lasting as that produced by small amounts plus small doses of toxine.

IMMUNITY NOT TRANSMITTED TO SECOND GENERATION.

Two female guinea pigs born of mother No. 6 (see p. 15), and from a litter of which the other members had shown marked resistance to diphtheria toxine, were placed with a male pig born of untreated parents. Each female had two young. These were given an L+ dose to determine whether the immunity was transmitted to the second generation (grandchildren). The results are given below:

Guinea pig No. 6ac. Given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15.VI .06. Died in 3 days 14 hours. Control died in 3 days 17 hours.

Guinea pig No. 6bc. Given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15.VI .06. Died in 3 days 20 hours. Control died in 3 days 14 hours.

Guinea pig No. 6ae. Given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15.VI. 06. Died in 4 days 4 hours. Control died in 3 days 17 hours.

Guinea pig No. 6be. Given 0.22 c. c. toxine No. 7 + 1 immunity unit Ehrlich 15.VI .06. Died in 3 days 17 hours. Control died in 3 days 14 dours.

From the above it seems plain that the resistance to diphtheria toxine is not transmitted to the second generation.

THE CUMULATIVE EFFECT OF DIPHTHERIA TOXINE.

An attempt was made to immunize female guinea pigs by repeated small injections of diphtheria toxine. Doses considerably less than the MLD and not sufficient to cause a perceptible reaction were given at intervals of about 7 days. Before an amount equal to an MLD had been given the animal in each case died. These results are in exact accord with the work of Behring and Kitashima,[a] who found that guinea pigs, when given daily injections of diphtheria toxine and when the total amount injected was only $\frac{1}{400}$ of an MLD, after a time died with the post-mortem lesions of poisoning from diphtheria toxine. Instead of being able to produce an immunity to the toxine a hypersusceptibility was produced.

A study of Tables 1 and 2 will show that in no case in which the preliminary treatment of the mother was of toxine alone was there an immunity transmitted by the mother to her young. This at first seemed to me a remarkable fact. I conceive the reason of the failure for this immunity to be transmitted by pigs whose preliminary treatment was toxine alone to be due to the fact that the mother pig was not immunized by a single dose of toxine, and that it is difficult to produce an active immunity in guinea pigs by the repeated injections of toxine, for before this result is obtained the cumulative effect of the toxine kills the animal.

It seems to me that instead of calling it a hypersusceptibility to the poison, as did Behring and Kitashima, it would be more proper to consider this action due to the cumulative effect of repeated small doses of the toxine.

It is well known among clinicians that one attack of diphtheria, rheumatism, or pneumonia predisposes to a subsequent one. This may more properly be considered due to an induced hypersusceptibility, whereas the repeated injections of toxine, as in the case of the guinea pigs, is a cumulative action.

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[a] Von Behring and Kitashima: Über Verminderung und Steigerung der ererbten Gift empfindlichkeit. Berl. klin. Woch., 1901, p. 157.

II.

MATERNAL TRANSMISSION OF IMMUNITY TO DIPHTHERIA TOXINE AND HYPERSUSCEPTIBILITY TO HORSE SERUM IN THE SAME ANIMAL.

We know (loc. cit., p. 14) that the young of female guinea pigs that have received either before or during pregnancy toxine-antitoxin mixtures are hypersusceptible to an injection of horse serum either normal or antitoxic. It therefore occurred to me that it would be very interesting to determine whether such young pigs were also immune to diphtheria toxine.

For this purpose it was necessary first to test the immunity against the MLD, and if the animal recovered to test its hypersusceptibility to horse serum. We know (loc. cit., p. 17) that treatment with toxine alone does not render a guinea pig susceptible to a subsequent injection of horse serum.

Guinea pigs born of a susceptible mother and from a litter of which the other members were immune to diphtheria toxine were also used to test the transmission of hypersusceptibility to horse serum. The results are as follows:

FAMILY NO. 1.

Mother guinea pig No. 4495. 103 days before the birth of young given 0.19 c. c. toxine No. 7 + 1 immunity unit B27. Local reaction, paralysis; recovered.

April 25, 1906. Nos. 4495A, 4495B, and 4495C born.

June 22. Nos. 4495A and 4495C given 0.007 c. c. toxine No. 7. No reaction third day. Controls died in 2 days 2 hours and 1 day 12 hours.

The results of this test showed a distinct immunity to diphtheria toxine.

June 26. Both of the above and 4495B, which had received no toxine, were given 2 c. c. normal horse serum. All three died in less than 45 minutes.

FAMILY NO. 2.

Mother guinea pig No. 4. (For treatment of mother see p. 14.)

June 22, 1906. Guinea pigs Nos. 4E, 4F, received $8\frac{1}{3}$ times the MLD (see p. 14). Both showed slight induration on the third day; recovered.

June 26. Guinea pigs Nos. 4E, 4F, and 4G were given 2 c. c. normal horse serum. All three died in less than 45 minutes.

FAMILY NO. 3.

Mother guinea pig No. 8. (For treatment of mother see p. 16.)

June 22, 1906. Guinea pig No. 8G given 8⅓ times the MLD. Showed slight induration on the third day; recovered.

June 26. Guinea pigs Nos. 8G and 8I given 2 c. c. normal horse serum. Both died in less than 45 minutes.

Four control pigs of about the same age and weight, born of untreated mothers, received 4 c. c.—twice the amount given the test pigs—of normal horse serum. None showed any inconvenience as a result of the injection.

It will be readily seen from the above that a mother can transmit to the same young two properties, viz, immunity to diphtheria toxine and hypersusceptibility to horse serum.

It is therefore very manifest that female guinea pigs that have received the toxine-antitoxin mixtures should not be used as breeders by those engaged in work with diphtheria antitoxin. On account of the transmitted immunity misleading results in the testing of antitoxin and toxine will be had. On account of the hypersusceptibility to the injection of horse serum pigs derived from such a source, when used to determine the presence of bacterial or toxic impurities may die, not from the presence of such impurities, but on account of a hypersusceptibility to horse serum transmitted from the mother.

(Continued from second page of cover.)

No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

No. 23.—Changes in the Pharmacopœia of the United States of America, eighth decennial revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

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No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

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Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 31.

M. J. ROSENAU, Director,

AUGUST, 1906.

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VARIATIONS IN THE PEROXIDASE ACTIVITY OF THE BLOOD IN HEALTH AND DISEASE.

BY

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JOSEPH H. KASTLE

AND

HAROLD L. AMOSS.

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No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

(Continued on third page of cover.)

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1906.

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VARIATIONS IN THE PEROXIDASE ACTIVITY OF THE BLOOD IN HEALTH AND DISEASE.[a]

By J. H. Kastle,

Chief of the Division of Chemistry, Hygienic Laboratory, Public Health and Marine-Hospital Service.

and

Harold L. Amoss,

Assistant in the Division of Chemistry, Hygienic Laboratory, Public Health and Marine-Hospital Service.

It is to Schönbein[b] that we are indebted for the observation that aqueous extracts of various plant and animal tissues have the power of actively decomposing hydrogen peroxide and of greatly accelerating oxidations by means of this substance. It was shown, for example, that when a freshly prepared extract of malt is added to water containing small amounts of hydrogen peroxide and tincture of guaiacum the solution becomes dark blue in color, owing to the oxidation of the guaiacum. So delicate is this reaction that for many years it afforded the most sensitive test for hydrogen peroxide known to chemists. It was also observed by Schönbein[c], during the progress of these investigations, that blood corpuscles and ferrous salts have the power of rendering active the oxygen of hydrogen peroxide toward such oxidizable substances as guaiacum, indigo, and a mixture of potassium iodide and starch; and Hiss[d] pointed out that the activity of blood corpuscles in this regard diminishes in proportion to the withdrawal of iron therefrom. Schönbein, therefore, reached the conclusion that blood corpuscles owe their power to render active the oxygen of hydrogen peroxide to the iron which they contain, and in this connection he cited the interesting observation that blood corpuscles do not lose their power of rendering oxygen active, either through heating to boiling, or as the result of partial putrefaction. This would seem to indicate, as pointed out by Schönbein, that their power to render active the oxygen of hydrogen peroxide depends less on their organization as living cells than on their iron content.

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[a] Manuscript submitted July 5, 1906.

[b] Bericht über die Verhandlungen der Naturforschenden Gesellschaft in Basel, III, 697–721.

[c] Ibid. II, 9–15 (see also Jour. f. Prakt. Chem., Vol. 75, p. 73).

[d] Private communication to Schönbein.

Schönbein[a] made the further interesting observations that dried blood corpuscles are more active than fresh in the production of guaiacum blue from a mixture of guaiacum and hydrogen peroxide, and that hydrocyanic acid retards these oxidations in much the same way that it hinders alcoholic fermentation and the sprouting of seeds. He arrived at the conclusion, therefore, that hydrocyanic acid kills animals so rapidly for the reason that it hinders catalysis and interferes with respiration.

Aside from their general interest the chief importance attaching to these earlier observations of Schönbein is that these reactions afforded delicate tests both for hydrogen peroxide and blood; and of the various tests for blood[b] that have been proposed from time to time, certainly the greater number, if not all, depend on the production of some colored substance, as the result of the oxidation of a chromogenic substance by hydrogen peroxide and blood.

During recent years, however, the oxidizing ferments of the blood have been the subject of further investigations. Lumière and Chevrotier,[c] for example, have prepared a protoplasmic extract of red corpuscles by vigorously centrifugalizing a mixture of blood and isotonic liquid, collecting the corpuscles, and washing several times with the isotonic liquid. The washed corpuscles were then subjected to alternate freezing and thawing in order to disintegrate them, and the mixture centrifugalized. The liquid was then filtered and rendered isotonic by means of sodium chloride. It was again filtered and preserved in sterilized flasks. This liquid, which the authors have called hæmoplase, rapidly loses oxygen in vacuum, becoming violet black in color, the red color being restored by agitation with air. It was found by these observers to possess the properties of an oxidase to a very marked degree.

Quite recently, Jolles and Oppenheim[d] have made a quantitative study of the power of the blood of man and certain of the lower animals to decompose hydrogen peroxide. It has been found as the result of these investigations, that the blood of amphibious animals decomposes hydrogen peroxide very slowly as compared with the rate of decomposition brought about by the blood of man and the higher animals, and that the blood of fishes possesses the least catalytic activity of any blood thus far examined. It has also been observed that the power of the blood of man to decompose hydrogen peroxide suffers a marked diminution in certain pathological conditions such as tuberculosis, nephritis, and cancer. They have found further that the catalase of blood is destroyed by boiling, and also by acids; that it is present exclusively in the formed elements of the blood, and that roughly, at least, it is proportional to the amount of hemoglobin.

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[a] Bericht über die Verhandlungen der Naturforschenden Gesellschaft in Basel, IV, 410 and 767.

[b] Schaer, Zeit. f. anal. Chem., 1903, p. 1; and Archiv der Pharmacie, 238 (1900), 42–48.

[c] Compt. rend., 141 (1905), 142–143.

[d] Zeit. f. anal. Chem., 44 (1904), 1–5; and Münch. Med. Woch., 51, 2083–2085; and also Virchow's Archiv (1905), 180, 185 and 225.

Sometime ago, it was observed by one of us (Kastle) that the yellowish filtrate from boiled blood has the power of effecting the oxidation of phenolphthalin in alkaline solution, whereas, solutions of blood salts, with and without iron, prepared according to the analyses given in Bunge's Physiologic and Pathologic Chemistry (pp. 212–213), do not possess this property. Up to the present, we have not had an opportunity to investigate this interesting difference in conduct of these two solutions. It led to the notion, however, that it would probably be of interest to determine the oxygen-carrying power of the blood in alkaline solution under various conditions of health and disease, using phenolphthalin as the oxidizable substance and hydrogen peroxide as the oxidizing agent. In other words, the attempt has been made to determine what may be called the peroxidase activity of the blood of man under various conditions of health and disease.

THE BLOOD AS AN OXYGEN-CARRIER IN THE OXIDATION OF PHENOLPHTHALIN BY HYDROGEN PEROXIDE.

THE REACTION AND MODE OF PROCEDURE.

Phenolphthalin or dioxytriphenylmethane carbonic acid is the leuco compound of phenolphthalein. On oxidation, it passes into phenophthalein in the sense of this equation:

$$C_{20}H_{16}O_4+O=C_{20}H_{14}O_4+H_2O.$$

Hence, if the oxidation takes place in alkaline solution, we have the production of a deep purplish red compound (phenolphthalein in alkali) from a colorless salt. The oxidation of phenolphthalin may be accomplished by various peroxides, such as lead and manganese peroxide, benzoyl peroxide, etc., and by the plant oxidases. In fact, Kastle and Shedd[a] several years ago proposed a neutral solution of this compound as a reagent for the plant oxidases. Phenolphthalin is also slowly oxidized by hydrogen peroxide in neutral and alkaline solution. As is the case with many of the oxidations of this character, however, the change is greatly accelerated by various oxygen-carriers, such as platinum black, etc., and as indicated in the foregoing, by the peroxidase of blood. It will be seen from our results, which are given in the following pages, that when allowed to stand alone an alkaline solution of phenolphthalin and hydrogen peroxide gives only a faint pink coloration. If, however, a very small quantity of blood be added to such a solution it soon acquires the deep, purplish red coloration, characteristic of phenolphthalein in alkali, indicating that under the influence of blood or an oxygen-carrier, hydrogen peroxide is able to effect the oxidation of phenolphthalin with far greater rapidity than when allowed to react alone.

That a certain amount of free alkali is essential to this change may be gathered from the following: Six solutions were prepared, each containing 2 c. c. of fresh blood solution (Porch, normal), 1 cu. mm. to 250 c. c. and 5 c. c. of a reagent, containing 0.032 gram phenolphthalin, 0.0034 gram hydrogen peroxide, and 1 c. c. N 10 sodium hydroxide, the whole diluted to 100 c. c. To three of the mixed solutions 1 c. c. of water was added. These three solutions were labeled A. To each of the remaining solutions 1 c. c. of N/10 sodium hydroxide was added. These solutions containing the excess of sodium hydroxide were labeled B. One set of the solutions A and B was then allowed to stand for fifteen minutes at room temperature, 30° C., at the end of which interval 1 c. c. of N/10 sodium hydroxide was added to A and 1 c. c. of water was added to B. The depth of color of each was then determined by comparison with standard phenolphthalein placed at five divisions on the colorimeter scale, immediately and fifteen minutes after the final addition of sodium hydroxide and water with the following results:

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[a] Amer. Chem. Jour., 26 (1901), 526-539.

15-minute series.

READINGS ON COLORIMETER.

Time.	A.	B.
Immediately	[a] 175.	5
15 minutes	10.8	[b] 4.6

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[a] It should be borne in mind that a large number on the scale of the colorimeter (Duboscq-Pellin) signifies a faint coloration and the presence of a small amount of the colored substance, whereas, conversely, a small number on the colorimeter scale indicates a greater depth of color and a correspondingly larger amount of colored substance. For further details regarding this method of making the color comparisons see footnote *b*, p. 15, of this communication.

[b] B of course had stood altogether 30 minutes in contact with 1 c. c. N/10 sodium hydroxide, at a total dilution of 8 to 9 c. c.

A second pair of solutions A and B was allowed to stand in glass-stoppered bottles at 30° C. for two hours, at the end of this time 1 c. c. of N/10 sodium hydroxide was added to A and 1 c. c. of water to B. The depth of color of each was then determined by comparison with a standard solution of phenolphthalein solution in alkali in the colorimeter, the standard tube of the colorimeter being placed at 5 divisions on the scale, immediately and after standing fifteen minutes, with the following results:

Two-hour series.

READINGS ON COLORIMETER.

Time.	A.	B.
Immediately	178	6.7
15 minutes	3.9	6.7

The third set of solutions was allowed to stand for three hours at ordinary temperature, at the end of which time 1 c. c. of N/10 sodium hydroxide was added to A and 1 c. c. of water to B. The color intensity of both solutions was then determined immediately in the manner already described with the following results:

Three-hour series.

READINGS ON COLORIMETER.

Time.	A.	B.
Immediately	235	7.6
15 minutes	65	7.5

It is evident from these results that the oxidation of phenolphthalin by blood and hydrogen peroxide takes place only in alkaline solution, and a comparison of the 15-minute, two, and three hour series with one another goes to show that on standing in contact with hydrogen peroxide and the sodium salt of phenolphthalin at the concentration indicated above, the blood loses a great deal of its oxygen-carrying power at the same time that it probably effects the decomposition of the hydrogen peroxide catalytically. (See the work of Jolles and Oppenheimer.) With the view of throwing still further light on these points, 2 c. c. of blood solution (1 cu. mm. blood to 250 c. c.) were added to solution A of the three-hour series and to solution B of the same series 2 c. c. of distilled water. These solutions were then allowed to stand at ordinary temperature and at the end of fifteen minutes and one hour the depth of color of each determined in the colorimeter in the manner already described with the following results:

READINGS ON COLORIMETER.

Time.	A.	B.
15 minutes	12.7	8.6
1 hour	6	9

Comparing the numbers under A with those given under A in the three-hour series, we note a remarkable increase in the depth of color and hence an increase in the amount of phenolphthalin oxidized, as the result of adding the second 2c. c. of blood solution, thereby showing that while all other substances needful to effect the oxidation of the phenolphthalin were at hand in solution A of the three-hour series the oxygen carriers of the first portion of blood solution had been so altered by standing in contact with hydrogen peroxide and the sodium salt of phenolphthalin as to become largely incapable of effecting the oxidation.

It has also been observed that within certain limits the oxidation of phenolphthalin by blood and hydrogen peroxide is nearly proportional to the concentration of the sodium hydroxide. That such is the fact may be seen from the following: Two solutions were prepared and labeled (1) and (2). (1) contained 0.032 gram phenolphthalin, 0.0034 gram hydrogen peroxide, 30 c. c. N 100 sodium hydroxide, the whole made up to 100 c. c. with distilled water. (2) was the same as (1) except that it contained 21 c. c. N 10 sodium hydroxide. Bearing in mind that 10 c. c. N 100 or 1 c. c. N 10 sodium hydroxide are required respectively to neutralize the phenolphthalin present in these two solutions, it will be seen that the free alkali in (1) and (2) corresponds to 20 c. c N 100 and 20 c. c. N 10 sodium hydroxide respectively. In order to determine the effect of alkali at these two concentrations on the oxidizing power of blood, 5 c. c. of solutions (1) and (2) were taken and 2 c. c. of a normal blood solution, 1 cu. mm. to 250 c. c., added to each. The mixtures were then allowed to stand for the several intervals indicated below at the end of each of which the depth of color of each solution was determined by comparison with a standard solution of phenolphthalein in alkali by means of the colorimeter, the standard being set at 5 divisions on the colorimetric scale:

READINGS ON COLORIMETER.

Time.	(1)	(2)
1 hour	17.1	1.9
24 hours	17.25	5.6
48 hours	18	4.1

It is evident, therefore, that the amounts of phenolphthalin oxidized under these conditions are, for a short interval of time, nearly proportional to the concentration of the sodium hydroxide.

It should also be borne in mind that in alkaline solution, blood alone has the power of effecting the oxidation of phenolphthalin, and that this oxidizing power is not completely destroyed by boiling, although it is considerably weakened. Were this direct oxidizing power of the blood completely destroyed by heat, we should be inclined to refer its oxidizing power to the presence of an oxidase. If the oxidizing substance is really an oxidase, we must look upon it as possessing far greater stability than the plant and animal oxidases generally. Whether the oxidation of phenolphthalin by blood in alkaline solution in the presence or absence of hydrogen peroxide is referable to the same cause will be the subject of further investigation. It is sufficient for present purposes to bear in mind that at the dilution of blood employed in making these measurements of peroxidase activity—viz, 1 cu. mm. of blood to 250 c. c.—the direct oxidizing power of the blood in alkaline solution is extremely feeble, whereas, in the presence of hydrogen peroxide the oxidation of the phenolphthalin proceeds with considerable velocity.

MODE OF PROCEDURE.

It was found, as the result of a few preliminary observations, that in determining the peroxidase activity of the blood by this method, the greatest care would have to be exercised in order to obtain reliable and comparable results. Such being the case, the following mode of procedure was agreed upon: In the first place, the work was so arranged that the collection of samples of blood, together with the determination of the amount of hemoglobin in the several samples, was left to Amoss, whereas the chemical measurements and also the preparation of the reagents required devolved upon the other party concerned in this investigation. By this arrangement it was hoped to secure greater accuracy than could otherwise be obtained.

In collecting the samples of blood, the following mode of procedure was agreed upon and rigorously adhered to. In nearly all cases the samples of blood were obtained from the lobe of the ear. When this was impracticable, from the tip of the fore finger. The part was gently stroked for some time in order to flush it with blood. A deep stab was then made with a blood lancet and two samples collected, one for the hemoglobin determination, using a Dare hemoglobinometer; the second was carefully collected in a 1 cu. mm. blood pipette, which was then carefully washed out with a small amount of distilled water, and the sample made up to 5 c. c. with distilled water. The determination of the peroxidase activity of the second sample was then made as soon as possible; never after a longer interval than three hours, except in those cases where it was desired to determine the effect of standing in contact with water on the peroxidase activity. The peroxidase activity of the several samples of blood was determined in the following manner:

The solution of blood in 5 c. c. of water was carefully poured into a 250 c. c. measuring flask, the bottle washed, and the solution exactly made up to 250 c. c. with distilled water. 2 c. c. portions of this more dilute solution were then accurately measured out, using a form of pipette described by Rosenau[a] in his work on the determination of the immunity unit for standardizing diphtheria antitoxin and which, for such work as this now under consideration, possesses many advantages in point of accuracy over the older method of using the pipette. These 2 c. c. portions of the blood solution (1 cu. mm. blood to 250 c. c.) were then mixed with 5 c. c. of a reagent containing the following substances: 0.032 grams phenolphthalin, 21 c. c. N/10 sodium hydroxide, 10 c. c. hydrogen peroxide, containing .034 grams H_2O_2 in 100 c. c., the whole made up to 100 c. c. with distilled water. The mixtures of blood solution and the reagent were then preserved in glass-stoppered bottles for the desired intervals, usually one to twenty-four hours. During this time the solutions acquired the purplish red color of phenolphthalein in alkali, and at the end of the given interval the amount of phenolphthalein produced as the result of the oxidation of the phenolphthalin was determined by comparing the color of the solution with that of a standard solution of phenolphthalein in alkali containing 0.000318 grams of phenolphthalein, and 1 c. c. N/10 sodium hydroxide at a total dilution of 7 c. c. The color comparisons were made in a Duboscq-Pellin colorimeter, the readings being made in diffused light.

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[a] Bulletin 21, Hygienic Laboratory, April, 1905, fig. 13, p. 64.

That the amount of phenolphthalein formed as the result of the oxidation of phenophthalin can be accurately determined by this colormetric method, provided an excess of alkali, not too great, is present in the solution, may be seen from the following observation:

A solution of phenolphthalein was prepared containing 0.159 grams of the substance in 100 c. c. of absolute alcohol. One cubic centimeter of this solution, which we will call solution A, was mixed with 5 c. c. of water and 1 c. c. N 10 sodium hydroxide. The color of this solution was then compared in the colorimeter with that of a standard solution of phenolphthalein containing 0.0318 grams of the compound in 100 c. c. of absolute alcohol, using the same quantity of water and sodium hydroxide. These solutions gave the following readings on the colorimeter:

Solution A.	Standard.
1.1	5
1	5

The theory for these solutions is 1:5, inasmuch as the stronger solution contains five times as much phenolphthalein as the standard.

On account of the very slight solubility of phenolphthalein in water, considerable difficulty has been encountered in the preparation of the standard solution of phenolphthalein with which to make the color comparisons. Hence it is, that during the progress of the investigation, several such standard solutions of phenolphthalein have been employed, so that, in the majority of cases, at least, the results obtained in the several series of experiments are not strictly comparable, one series with another, but only the results of any particular series among themselves.

Another reason why the results of any particular series of experiments are only comparable among themselves and not with those of any other series is that the oxidation of phenolphthalin by hydrogen peroxide under the accelerating action of blood is like the greater number of chemical processes influenced by temperature; the rate of oxidation increasing, within certain limits at least, with rise of temperature, this effect being most noticeable for short intervals of time. That such is the case may be seen from the following observations:

Solutions were prepared containing 2 c. c. of the dilute solution of Amoss' blood (normal), containing 1 cu. mm. of blood in 250 c. c. and 5 c. c. of the reagent. Glass-stoppered bottles containing these solutions were kept at 2° C., 14° C., and 39° C., for fifteen minutes and one hour, respectively, at the end of which time the color of the several solutions was compared with that of a standard solution of phenolphthalein in N 10 sodium hydroxide. The standard was arbitrarily set at 5 divisions on the scale of the colorimeter. The following readings were obtained for these several solutions at the temperatures and intervals indicated above:

Time.	Temperature.	Readings on colorimeterscale.	Standard.
	°C.		
15 minutes	2	10.5	5
Do	14	4.6	5
Do	39	3.1	5
1 hour	2	3.8	5
Do	14	2.8	5
Do	39	3.6	5

It will be observed, therefore, especially for the shorter interval, that a rise of temperature causes an increase in the amount of phenolphthalin oxidized, the apparently low result reached at 39° C. for one hour being due to a gradual fading out of color of the phenolphthalein in alkali, a change which also probably takes place more rapidly with rise of temperature and which will be more fully discussed in a subsequent portion of this communication. Up to this time our experiments have been carried on at room temperature which has varied within a range of ten degrees or more, during the time that this investigation has been in progress. Hence, we see that only the results of any one series of experiments are strictly comparable among themselves, for the reason that it has proven practicable to carry out only one series of experiments at any particular time and day.

Up to the present time the peroxidase activity of the blood of forty-seven different persons has been determined in the manner above described with the following results:

SERIES NO. 1.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 1,[a] set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.[b]	Quantity of phenolphthalin oxidized, in milligrams.	Per cent oxidized	Oxidizing power of the blood, in terms of normal = 100.
Apr. 22	Amoss	Normal	96	3.4	0.468	0.471	29.4	100
22	Collier	Pernicious anemia	20–21	19.5	.0816	.082	5.1	17
22	Hynson	do	27	11.6	.137	.138	8.6	29

SERIES NO. 2.

Apr. 24	Amoss	Normal	96	6.6	0.24	0.242	15.1	100
24	Callahan	Fibroma, uterus	31	21.75	.0731	.074	4.6	30.4
24	Johnson	Disease unknown	49	12.5	.127	.128	8	53

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[a] Standard phenolphthalein solution No. 1 contained 0.0318 gram phenolphthalein in 100 c. c. alcohol. 1 c. c. of this solution measured out by pipette in usual manner and mixed with 5 c. c. of distilled water and 1 c. c. N/10 sodium hydroxide furnished the standard for the color comparison.

[b] The quantity of phenolphthalein formed in milligrams and the quantity of phenolphthalin oxidized, also in milligrams, has been calculated in the following manner: As already pointed out, 5 c. c. of the phenolphthalin reagent was used throughout the work. This reagent contained 0.032 gram of phenolphthalin in 100 c. c. of the solution; 1 c. c. of the reagent contained 0.00032 gram of phenolphthalin, and 5 c. c. contained 0.00032×5=0.0016 gram of phenolphthalin. If this were completely oxidized, it would yield 0.00159 grams of phenolphthalein. The standard solutions of phenolphthalein employed in making the color comparisons all contained 0.0318 gram of phenolphthalein in 100 c. c. of solution. Hence, 1 c. c., the amount employed in making up the standard for the color comparison, contained 0.000318 gram of phenolphthalein or one-fifth of the amount of phenolphthalein which would have been formed had all of the phenolphthalin employed in each test been oxidized. In making the color comparisons in the colorimeter, the colorimeter scale on the side of the standard was arbitrarily placed at 5 divisions and the other scale adjusted until the colors matched on the two sides of the instrument. If, now, in order to match the standard, which had been set at 5, the other scale should have had to be placed at 5, then the color of the solution originally would have been exactly equal to that of the standard or to the color produced by 0.000318 gram of phenolphthalein, or 0.318 milligram of phenolphthalein would have resulted from the oxidation. Had it been necessary to set the scale of the test at 1 in order to match the standard at 5, then, obviously, five times the amount of phenolphthalein present in the standard, or 0.000318×5=0.00159 gram, or 1.59 milligrams, would have been formed.

Hence, in order to calculate the amount of phenolphthalein formed during these experiments we have from the foregoing the following proportions:

$$5 : 5 :: .000318 : x$$

and

$$1 : 5 :: .000318 : x$$

from which it follows that the amount of phenolphthalein formed is equal to 0.00159 divided by the reading on the colorimeter scale, when the standard is set at 5 on the same scale, and since

$$318 : 320 :: 1 : 1.006$$

we multiply the number of milligrams of phenolphthalein formed by 1.006 in order to get the amount of phenolphthalin oxidized in milligrams.

To take the first case under Series No. 1, April 22, the colorimeter reading was 3.4 in terms of the standard=5. Hence, to calculate the amount of phenolphthalein formed, we would have:

$$\frac{0.00159}{3.4}=.000468 \text{ or } 0.468 \text{ milligrams}$$

and for the phenolphthalin oxidized:

$$0.468\times1.006=0.471.$$

(See Series No. 1, Apr. 22.)

SERIES NO. 3.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 2,[a] set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.	Quantity of phenolphthalein oxidized, in milligrams.	Per cent oxidized	Oxidizing power of blood, in terms of normal = 100.
Apr. 25	Amoss	Normal	107	4.6	0.346	0.348	21.8	100
25	Edna	Broncho-pneumonia	28	25	.064	.0644	4.02	19
25	Thomas	Disease unknown	42	13.6	.117	.118	7.4	34
25	Antony	do	22	31.5	.0505	.0508	3.2	15
25	Teddy	do	44	13.2	.121	.122	7.6	35

SERIES NO. 4.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 2,[a] set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.	Quantity of phenolphthalein oxidized, in milligrams.	Per cent oxidized	Oxidizing power of blood, in terms of normal = 100.
Apr. 26	Amoss	Normal	107	3.8	0.42	0.423	26.4	100
26	Carl	Disease unknown	43	9.2	.173	.177	11.1	42
26	Irvine	do	82.5	4.4	.361	.363	22.7	86
26	T. Tanner	Slight anemia	75	5.1	.311	.313	19.6	74

[a] Standard phenolphthalein solution No. 2 same as No. 1, except that the solution was made up with water and alcohol. On standing it deposits phenolphthalein, and hence can not be kept for more than a few days at a time.

SERIES NO. 5.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 3,[a] set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.	Quantity of phenolphthalein oxidized, in milligrams.	Per cent oxidized.	Oxidizing power of blood, in terms of normal = 100.
May 3	Amoss	Normal	105	2.65	0.6	0.604	37.8	100
3	Seboure	do	100	2.5	.636	.64	40	105
3	Beavers	do	88	3	.53	.533	33.3	88
3	Porch	do	92	2.8	.569	.571	35.6	94
3	Murray	do	86	3	.53	.533	33.3	88
3	Schrader	do	90	2.8	.568	.571	35.6	94

SERIES NO. 6.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 3,[a] set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.	Quantity of phenolphthalein oxidized, in milligrams.	Per cent oxidized.	Oxidizing power of blood, in terms of normal = 100.
May 7	Amoss	Normal	97	2.1	0.757	0.762	47.6	100
7	Comber	Fibroma, uterus	54	4	.398	.401	25.1	53
7	Cline	Typhoid fever	77	3.5	.454	.457	28.5	59.5
7	Carrolli	Empyema	79	3.3	.481	.484	30.2	63

[a] Standard phenolphthalein solution No. 3 same as No. 2, except that it contained a larger proportion of alcohol. It also deposited phenolphthalein after long standing.

SERIES NO. 7.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 4,[a] set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.	Quantity of phenolphthalein oxidized, in milligrams.	Per cent oxidized	Oxidizing power of blood, in terms of normal =100.
May 9	Geo. Bunay	Typhoid fever	88	6.1	0.26	0.262	16.4	70
9	R. Somax	Typhoid fever convalescent.	73.5	7.5	.212	.213	13.3	57
9	Ger. Rossier	Hemorrhage from miscarriage.	67	6.3	.252	.254	15.9	67
9	R. Russ	Pelvic cyst	83	5	.318	.32	20	86
9	L. Grant	Suspected tuberculosis.	66	6.4	.248	.25	15.6	66
9	G. Saunders	Menorrhagia	67	5.9	.27	.272	17	73
9	R. Spencer	Disease of kidneys	100	3.4	.467	.47	29.4	126
9	M. Foster	General sepsis	52	9.4	.169	.17	10.6	45.5
9	Amoss	Normal	97	4.3	.37	.372	23.3	100

SERIES NO. 8.

May 10	McIntyre	Tuberculosis and heart disease.	84	4.8	0.331	0.333	20.8	92
10	D. Murphy	Typhoid fever	80	5.6	.283	.285	17.8	79
10	G. Corlee	Acute rheumatism	61	7.3	.218	.219	13.7	61
10	Mrs. Aley	Tuberculosis	79	5.45	.291	.293	18.3	81
10	Amoss	Normal	97	4.45	.357	.359	22.4	100

SERIES NO. 9.

May 22	L Shepherd	Healthy child	90	6.4	0.248	0.25	15.6	74.3
22	Mrs. Shepherd	Not normal	84	5.5	.29	.292	18.2	86.6
22	Mrs Kastle	Normal	91	4.7	.338	.34	21.2	101
22	Dr. Shepherd	Not normal	79	6.2	.256	.258	16.1	76.6
22	Amoss	Normal	100	4.75	.335	.337	21	100
22	Water control		None.	([b])	None.	None.	None.	None.

SERIES NO. 10.

June 4	R. Hickey	Wound in knee	99	4.2	0.38	0.382	23.9	110
4	S. Lafero	Abdominal cyst	86	6.6	.241	.243	15.2	70
4	J. Reeves	Uterine fibroma	35	19.3	.082	.0825	5.1	23.5
4	Alice Kinsley	Shingles	81	5.5	.29	.292	18.2	83.8
4	E. Mason	Tuberculosis	71	7.3	.22	.221	14.8	68.2
4	Amoss	Normal	90	4.6	.345	.347	21.7	100
4	Amoss (June 2)	do	99	4.7	.328	.34	21.2	97.7

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[a] Standard phenolphthalein solution No. 4 was prepared by dissolving 0.0318 gram of phenolphthalein in absolute alcohol and making up to 100 c.c. with absolute alcohol; 1 c.c. of the solution measured out in pipettes by Rosenau's method and mixed with 5 c.c. water and 1 c.c. N/10 sodium hydroxide gave the standard for the color comparison, and was employed in all subsequent parts of the work, unless expressly stated to the contrary.

[b] Colorless.

SERIES No. 11.

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of 24 hours compared with standard phenolphthalein solution No. 4, set at 5 divisions on the colorimeter scale.	Quantity of phenolphthalein formed, in milligrams.	Quantity of phenolphthalein oxidized, in milligrams.	Per cent oxidized.	Oxidizing power of blood, in terms of normal =100.
June 5	M. Rawles.......	Child 10 days old.....	87	7.3	0.22	0.221	14.8	82.2
5	K. Riley	Hemorrhoids and abdominal cyst.	70	13.1	.121	.122	7.5	42.2
5	G. Bunay........	Typhoid convalescent	89	8.6	.185	.186	11.6	64.4
5	Wm. Pryor......	Tuberculosis of hip joint.	36	20.6	.077	.0775	4.84	27
5	Ellen Lehr......	General tuberculosis .	49	9	.177	.178	11.1	61.6
5	Amoss	Normal................	86	5.6	.284	.286	18	100

It is evident, from these results, that in diseased conditions the blood shows a considerable falling off in peroxidase activity, or oxygen-carrying power, as compared with that in normal conditions of health, and that in the majority of instances, at least, the peroxidase activity of blood is proportional to the percentage of hemoglobin. That such is the case may be seen by comparing the numbers given in the last column of our tables, in which the oxidizing power of the several specimens of blood are given in terms of normal blood (Amoss), the oxidizing power of which has arbitrarily been made equal to 100, with the several percentages of hemoglobin which are to be found in another column of the tables. It will be observed that in 29 out of the 47 specimens examined the numerical agreement between the oxidizing power of the blood and the percentage of hemoglobin is almost numerically exact, and that in the remaining cases it is sufficiently close to indicate a general agreement and correlation between oxidizing power and hemoglobin content.

The greatest differences in oxidizing power of the several samples of blood were brought out by allowing the oxidation to proceed for twenty-four hours. In most cases, however, if not in all, differences of a similar nature were shown after much shorter intervals. Thus, with the bloods composing series No. 1, which were examined on April 22, the following results were obtained for several intervals, three of which were shorter than twenty-four hours. (See Table No. 1.)

TABLE No. 1.

[Series No. 1 (for several intervals).]

Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of intervals indicated compared with standard phenolphthalein solution, set at 5 divisions on the colorimeter scale.				Quantity of phenolphthalein formed, in milligrams, during the several intervals.			
				5 minutes.	15 minutes.	1 hour.	24 hours	5 minutes.	15 minutes.	1 hour.	24 hours.
Apr. 22	Amoss..	Normal.............	96	8.75	3.3	2.6	3.4	0.182	0.481	0.612	0.468
22	Collier..	Pernicious anemia.	20–21	16.5	8.8	8.45	19.5	.096	.181	.188	.0816
22	Hynson.	.....do...............	27	16.5	7.2	6.1	11.6	.096	.221	.261	.137

Date.	Name of person.	Quantity of phenolphthalin oxidized, in milligrams, during the several intervals.				Percentage of oxidation during the several intervals.			
		5 minutes.	15 minutes.	1 hour.	24 hours.	5 minutes.	15 minutes.	1 hour.	24 hours.
Apr. 22	Amoss....	0.183	0.484	0.616	0.471	11.4	30.2	38.5	29.4
22	Collier....	.097	.182	.189	.082	6	11.3	11.8	5.1
22	Hynson...	.097	.222	.263	.138	6	14	16.4	8.6

FADING OF THE PHENOLPHTHALEIN COLOR.

Reference has already been made to the fact that the color of the phenolphthalein solution resulting from the oxidation of phenolphthalin by hydrogen peroxide under the influence of minute quantities of blood gradually fades somewhat, so that apparently the quantity of phenolphthalin oxidized at the end of twenty-four hours is less than the amount oxidized during one hour.

That such is the case is evident from the results given in Table No. 1. An examination of these results shows an increase in the amount of oxidation up to the end of one hour, after which a gradual fading of the color of the solution occurs until, at the end of twenty-four hours, the amount of phenolphthalin oxidized is apparently from one-quarter to one-half less than the amount oxidized at the end of one hour. Without it is that the blood contains a substance capable of reducing phenolphthalein—and this, of course, is conceivable—it is difficult to see why less phenolphthalin should be oxidized at the end of twenty-four hours than at the end of one hour. As a matter of fact the fading out of the phenolphthalein color seems to depend upon several causes.

In the first place, it seems to depend upon the character of the blood, being greater and more rapid in the case of diseased bloods, poor in hemoglobin. The result is that the most striking differences between normal and diseased bloods are revealed after the longer interval of time, this condition apparently resulting from a difference in oxidizing power in the first place, and also from a difference in the conduct of the blood toward phenolphthalein in alkali after this substance has been once formed.

In the second place, as is well known, a large excess of alkali destroys the colored substance first formed by the action of alkali on phenolphthalein by the formation of a colorless salt. The rate of decomposition of the colored compound of phenolphthalein and alkali seems to depend entirely on the concentration of the alkali. With large amounts of alkali the colored compound is destroyed immediately; with greater dilution and smaller amounts of alkali the colored compound is decomposed more slowly and gradually. That it gradually disappears from such a solution, however, may be seen from the following observations.

In order to determine the effect of increasing amounts of sodium hydroxide on the color of phenolphthalein, five solutions were prepared, each containing 0.318 milligram of phenolphthalein in 1 c. c. of absolute alcohol and 1, 2, 3, 4, and 5 c. c. N 10 sodium hydroxide, together with sufficient water to make the total volume up to 7 c. c. in each case. The color of these solutions was then compared with that of a phenolphthalein standard, containing 0.318 milligram in 1 c. c. absolute alcohol, 1 c. c. N 10 sodium hydroxide, and 5 c. c. of water, the standard being set at 5 divisions on the colorimeter scale.

The following readings were obtained for the several solutions immediately and after twenty-four hours, the comparison being made with freshly prepared standard in each case.

READINGS ON COLORIMETER.

Quantity of sodium hydroxide N/10	Immediately.	After 24 hours.	Standard.
c. c.			
1	4.9	8.7	5
2	4.95	16.1	5
3	5.4	22.75	5
4	Lost.	Lost.	5
5	13.4	22.6	5

Even at a dilution five times as great as that employed in the above series of experiments, viz, with N 50 sodium hydroxide, the color of phenolphthalein in alkali shows a tendency to fade on standing, but by no means so rapid and pronounced as with N 10 sodium hydroxide. Five solutions were prepared, each containing 0.318 milligram phenolphthalein in 1 c. c. absolute alcohol, and 1, 2, 3, 4, and 5 c. c. N 50 sodium hydroxide and sufficient water to make the total volume of the several solutions up to 7 c. c. in each case. These were compared with a fresh standard solution of phenolphthalein containing 0.318 milligram of phenolphthalein in 1 c. c. of absolute alcohol, 1 c. c. N/10 sodium hydroxide, and 5 c. c. of water, immediately and after twenty-four hours, with the following results:

READINGS ON COLORIMETER.

Quantity of sodium hydroxide N/50.	Immediately.	After 24 hours.	Standard.
c. c.			
1	5	6	5
2	5	6.3	5
3	4.8	7.5	5
4	5	7.7	5
5	4.9	9.3	5

Similar experiments were tried using N/500 sodium hydroxide in the proportion of 1, 2, 3, 4, and 5 c. c. at a total dilution of 7 c. c. The following results were obtained on comparison with the phenolphthalein standard employed in the previous series of experiments:

READINGS ON COLORIMETER.

Quantity of sodium hydroxide N/500.	Immediately.	After 24 hours.	Standard.
c. c.			
1	12	Colorless.	5
2	6.5	8.8	5
3	5.5	6.4	5
4	5.5	5.9	5
5	5	5.9	5

It would seem, therefore, that, so far as the influence of alkali on the fading of the phenolphthalein color is concerned, the most rapid fading occurs either with very small or with very large amounts of alkali. With very small amounts of alkali the fading out of the color is doubtless due to the absorption of carbon dioxide from the air in sufficient amounts to decompose the colored salt, and in the case of larger amounts of alkali the fading is probably connected with the formation of a colorless salt-like body from the colored quinoid substance. Regardless of any theory of the process, the fact remains that the presence of alkali may be responsible for the fading out of the phenolphthalein color encountered in our own experiments with blood.

Still, a third factor involved in the fading of the phenolphthalein color is the presence of hydrogen peroxide. That such is the case, is indicated by the fact that in those experiments in which blood alone is employed to effect the oxidation of phenolphthalin in alkaline solution, the depth of color persists unchanged for a much longer interval of time than when hydrogen peroxide is present, and yet the other conditions of the experiments are essentially the same, except that slightly larger amounts of blood are employed in effecting the oxidation and slightly more phenolphthalein is formed as the result of the oxidation, and hydrogen peroxide is absent, or if present, only as a minute impurity in the reagents employed. Otherwise the general conditions of the experiments are the same in the two cases, and the same amount of sodium hydroxide is present at the same concentration.

It is more than likely, therefore, that the gradual fading out of the phenolphthalein color is not due entirely to any one of the three factors enumerated in the foregoing, but rather to a combination of causes, the complete effect of which it is impossible to picture at the present stage of this investigation.

Suffice it to say in this connection, however, that this phenomenon in nowise invalidates either our results or the deductions therefrom.

THE DIRECT OXIDATION OF PHENOLPHTHALIN BY BLOOD IN ALKALINE SOLUTION.

Reference has already been made to the fact that in alkaline solution phenolphthalin is directly oxidized by blood without the intervention of hydrogen peroxide. It therefore occurred to us to make a few measurements of the direct oxidizing power of a number of specimens of blood from different individuals with the view of noting any possible variations that might exist among the several specimens in health and disease.

Somewhat larger amounts of blood are required to effect the oxidation than when hydrogen peroxide is present. Accordingly in this series of experiments 1 cubic millimeter of blood was diluted to 100 c. c. with distilled water. Two cubic centimeters of this solution were then brought together with 5 c. c. of a reagent containing 0.032 gram of phenolphthalin and 21 c. c. N 10 sodium hydroxid at a total dilution of 100 c. c.

These mixtures were allowed to stand in glass-stoppered bottles for the several intervals of time recorded in Table No. 2, at the end of which the amount of phenolphthalein formed as the result of the oxidation was determined colorimetrically by comparison in the colorimeter with standard solution of phenolphthalein No. 4. The results of these measurements are given in Table No. 2.

TABLE No. 2.

Series.	Date.	Name of person.	Condition.	Per cent of hemoglobin.	Colorimeter readings at the end of intervals indicated compared with standard phenolphthalein solution, set at 5 divisions on the colorimeter scale.							
					1 hour.	2 hours.	24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	168 hours.
1	June 2	Amoss	Normal	99	21.8	....	1.60	1.55	1.6	2	....	....
		Porch	do	95	21.4	....	1.50	1.50	1.6	1.6	....	....
		Schrader	do	94	22	....	1.55	1.50	1.95	1.9	....	....
		Control (water)		....	(a)	....	....	(b)	....	(b)	....	....
2	June 4	Amoss (June 2)	Normal	99	....	16	1.7	1.5	1.7	2.1	2.7	3.6
		Amoss (June 4)	do	90	....	16	1.8	1.5	1.6	1.9	2.4	3.7
		Robt. Hickey	Fracture wound in knee.	99	....	15.8	1.8	1.5	1.7	1.7	2.5	3.1
		S. Lafero	Abdominal cyst	86	....	16	1.7	2.1	2.1	2.4	3	4.1
		J. Reeves	Uterine fibroma	35	....	27.4	3.7	3.3	4.3	4.8	6.7	8.9
		Alice Kinsley	Shingles	81	....	16.6	1.9	1.9	1.8	2.2	2.9	5
		Edith Mason	Tuberculosis	71	....	17.4	1.9	2.2	2.3	2.7	3.1	4.8
		Control (water)		....	....	(a)	(a)	(b)	(b)	....	....	....
3	June 5	Amoss	Normal	86	....	21	1.6	....	....	....	....	....
		M. Rawles	Child 10 days old	87	....	23.4	1.6	....	....	....	....	....
		K. Riley	Hemorrhoids and abdominal cyst.	70	....	28	2.1	....	....	....	....	....
		G. Bunay	Typhoid fever, convalescent.	89	....	26	1.9	....	....	....	....	....
		Wm. Pryor	Tuberculosis of hip joint.	36	....	42	2.9	....	....	....	....	....
		Ellen Lehr	General tuberculosis	49	....	33.4	2.4	....	....	....	....	....
4	June 6	Rowze	Fracture of skull	83	....	14	1.6	1.6	1.5	....	2.8	....
		M. Rowze	Labor—childbirth	54	....	19.2	1.9	2.2	2.5	....	4.2	....
		Amoss	Normal	90	....	14.2	1.55	1.55	1.6	....	2.5	....

Series.	Date.	Name of person.	Quantity of phenolphthalein formed, in milligrams, during the several intervals.							
			1 hour.	2 hours.	24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	168 hours.
1	June 2	Amoss	0.073	....	0.934	1.02	0.994	0.795	....	....
		Porch	.074	....	1.06	1.06	.994	.994	....	....
		Schrader	.072	....	1.02	1.06	.85	.840	....	....
2	June 4	Amoss (June 2)	....	0.099	.93	1.06	.93	.76	0.59	0.41
		Amoss (June 4)	....	.099	.88	1.06	.994	.84	.66	.43
		Robt. Hickey	....	.100	.88	1.06	.93	.93	.64	.51
		S. Lafero	....	.099	.93	.76	.76	.66	.53	.39
		J. Reeves	....	.058	.43	.48	.37	.33	.24	.18
		Alice Kinsley	....	.095	.84	.84	.88	.72	.55	.32
		Edith Mason	....	.091	.84	.72	.69	.59	.51	.33
3	June 5	Amoss	....	.076	.994	....	....	....	....	....
		M. Rowles	....	.068	.994	....	....	....	....	....
		K. Riley	....	.057	.76	....	....	....	....	....
		G. Bunay	....	.061	.84	....	....	....	....	....
		Wm. Pryor	....	.038	.55	....	....	....	....	....
		Ellen Lehr	....	.047	.66	....	....	....	....	....
4	June 6	Rowze	....	....	.994	.994	1.06	....	.56	....
		M. Rowze	....	....	.84	.72	.64	....	.38	....
		Amoss	....	....	1.02	1.02	.994	....	.64	....

(a) None.

(b) Very faint pink.

TABLE NO. 2—Continued.

Series.	Date.	Name of person.	Quantity of phenolphthalin oxidized, in milligrams, during the several intervals.							
			1 hour.	2 hours.	24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	168 hours.
1	June 2	Amoss	0.0735	....	1	1.03	1	0.81	....	....
		Porch	.075	....	1.07	1.07	1	1	....	....
		Schrader	.073	....	1.03	1.07	.86	.85	....	....
2	June 4	Amoss (June 2)	....	0.10	.94	1.07	.94	.77	0.60	0.45
		Amoss (June 4)	....	.10	.89	1.07	1	.85	.67	.44
		Robt. Hickey	....	.10	.89	1.07	.94	.94	.65	.52
		S. Lafero	....	.10	.94	.77	.77	.67	.54	.40
		J. Reeves	....	.058	.433	.49	.372	.332	.24	.18
		Alice Kinsley	....	.096	.85	.85	.89	.73	.56	.32
		Edith Mason	....	.092	.85	.73	.70	.60	.52	.332
3	June 5	Amoss	....	.077	1	....	....	....	....	....
		M. Rawles	....	.069	1	....	....	....	....	....
		K. Riley	....	.058	.77	....	....	....	....	....
		G. Bunay	....	.062	.85	....	....	....	....	....
		Wm. Pryor	....	.0382	.56	....	....	....	....	....
		Ellen Lehr	....	.0473	.67	....	....	....	....	....
4	June 6	Rowze	....	....	1	1	1.07	....	.56	....
		M. Rowze	....	....	.85	.72	.65	....	.382	....
		Amoss	....	....	1.03	1.03	1	....	.65	....

Series.	Date.	Name of person.	Percentage of oxidation during the several intervals.								Oxidizing power of blood in terms of normal. (Amoss)=100. (24-hour interval.)
			1 hour.	2 hours.	24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	168 hours.	
1	June 2	Amoss	4.6	......	62.5	64.4	62.5	50.6	....	....	100
		Porch	4.7	......	66.9	66.9	62.5	62.5	....	....	107
		Schrader	4.5	......	64.4	66.9	53.7	53.1	....	....	103
2	June 4	Amoss (June 2)	......	6.25	58.7	66.9	58.7	48.7	37.5	28.1	94
		Amoss (June 4)	......	6.25	55.6	66.9	62.5	53.1	42	27.5	89
		Rob't Hickey	......	6.25	55.6	69.9	58.7	58.7	40	32.5	89
		S. Lafero	......	6.25	58.7	48.7	48.7	42	33.7	25	91
		J. Reeves	......	3.61	27	30.6	23.2	20	15	11.25	43
		Alice Kinsley	......	6	53.1	53.1	55.6	45.6	35	20	85
		Edith Mason	......	5.75	53.1	45.6	43.7	37.5	32.5	20.7	85
3	June 5	Amoss	......	4.8	62.5	......	....	....	....	....	100
		M. Rawles	......	4.3	62.5	......	....	....	....	....	100
		K. Riley	......	3.6	48.7	......	....	....	....	....	78
		G. Bunay	......	3.9	53.1	......	....	....	....	....	85
		Wm. Pryor	......	2.4	35	......	....	....	....	....	56
		Ellen Lehr	......	2.9	42	......	....	....	....	....	67
4	June 6	Rowze	......	......	62.5	62.5	66.9	....	35	....	100
		M. Rowze	......	......	53.1	45.6	40	....	24	....	85
		Amoss	......	......	64.4	64.4	62.5	....	40	....	103

A consideration of these results shows that even though a relatively larger amount of blood is present in the solution, the oxidation proceeds much more slowly than when hydrogen peroxide is also present.

When hydrogen peroxide is present normal blood, at a dilution of

1 cu. mm. to 250 c. c., can oxidize 38.5 per cent of the phenolphthalin present in one hour; whereas blood alone, at a dilution of 1 c. mm. to 100 c. c. acting in the presence of alkali, is only able to oxidize 4.6 per cent of the phenolphthalin in the same time. When hydrogen peroxide is present the maximum of oxidation is reached in one hour; whereas in blood alone the maximum is reached only after twenty-four hours.

Secondly, as already pointed out in the foregoing, it is evident that when blood alone is employed to effect the oxidation of the phenolphthalin the depth and intensity of the phenolphthalein color persists unchanged for a much longer time than when hydrogen peroxide is present. For example, with normal blood the color remains undiminished for seventy-two hours or even longer; whereas with the blood of diseased persons the fading takes place somewhat more rapidly. On the other hand, it will be recalled in this connection that when hydrogen peroxide is present the color of the phenolphthalein fades considerably, even in twenty-four hours, and, in fact, often in even less time. Hence, as already pointed out, it would seem that one of the causes of the fading of the phenolphthalein color is the presence of hydrogen peroxide.

Another point of interest in this connection and one that requires still further investigation is that in no case here studied, either with or without hydrogen peroxide, is the phenolphthalin completely oxidized, the maximum oxidation with blood alone being 66.9 per cent and with blood and hydrogen peroxide 47.6 per cent. This either indicates an insufficiency in available oxygen or it may be associated with the fading of the phenolphthalein color.

Thirdly, it will be observed that the direct oxidizing power of the blood of fifteen persons has been determined for twenty-four hours. In the last column of the table will be found the oxidizing power of these several specimens of blood calculated in terms of normal blood (viz, that of Amoss), arbitrarily made equal to 100. A comparison of these numbers among themselves and with the percentages of hemoglobin of the several specimens reveals much the same relationship that has been found to exist between peroxidase activity and per cent of hemoglobin, viz, the former is proportionate to the quantity of hemoglobin present in blood. So here the higher the hemoglobin content the greater the direct oxidizing power of blood. Out of the fifteen specimens whose direct oxidizing power has been determined eight have been found to contain hemoglobin varying from 83 to 99, and eleven have been found to have a direct oxidizing power varying from 85 to 107. Four of these bloods were markedly low in percentage of hemoglobin, the quantity varying from 35 to 54 per cent and three have been found to have a low direct oxidizing power varying from 43 to 67.

As a general thing the numbers for the direct oxidizing power of these several specimens of blood in terms of normal blood (Amoss, equal to 100) are somewhat higher than the corresponding numbers for the percentages of hemoglobin. This is true in twelve out of the fifteen cases studied.

On account of the scarcity of clinical material we have not yet been able to subject this method to the rigorous test which is required before we shall be able to say whether such examinations as those herein described will have any practical value as aid to diagnosis. From the comparatively few cases, however, that we have thus far been able to collect there is every indication that such will prove to be the case. We propose, therefore, to extend these observations as rapidly as the necessary clinical material can be obtained and at the same time we shall endeavor to perfect and simplify the methods above described for determining the activity of the blood as a carrier of oxygen.

O

(Continued from second page of cover.)

No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

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No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

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No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxin. II. Maternal transmission of immunity to diphtheria toxin and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

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HYGIENIC LABORATORY.—BULLETIN No. 32.
M. J. ROSENAU, Director.
October, 1906.

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A STOMACH LESION IN GUINEA PIGS CAUSED BY DIPHTHERIA TOXINE

AND ITS BEARING UPON

EXPERIMENTAL GASTRIC ULCER.

BY

M. J. ROSENAU
AND
JOHN F. ANDERSON.

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(Continued on third page of cover.)

TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
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1906.

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A STOMACH LESION IN GUINEA PIGS CAUSED BY DIPHTHERIA TOXINE, AND ITS BEARING UPON EXPERIMENTAL GASTRIC ULCER.[a]

By Milton J. Rosenau,
Passed Assistant Surgeon, Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service,

and

John F. Anderson,
Passed Assistant Surgeon, Assistant Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

Guinea pigs dying acutely from injections of diphtheria toxine frequently show a lesion in the stomach which, so far as we know, has not been described.

Our attention was first called to the condition of the stomachs of guinea pigs used in our diphtheria work by Assistant Surgeon A. M. Stimson in the summer of 1905. Since that time we have collected from our autopsy records 2,882 protocols in which the condition of the stomach was noted. Of these, 1,897 guinea pigs, or 66 per cent, showed the lesion in the stomach described in this bulletin.

In the many thousand autopsies upon guinea pigs used in this laboratory in preparing the standard unit for measuring the strength of diphtheria antitoxin and also for verifying the potency of antidiphtheric serum made by the licensed manufacturers, we constantly see the lesions usually found in animals dying from diphtheria poisoning, such as darkened and congested adrenals, effusions into the pleura and other serous cavities, local edema, necrosis and hemorrhagic reaction at the site of inoculation, etc.; but it is difficult to understand how the condition of the mucosa of the stomach, which is so very evident in a large proportion of the guinea pigs, has so long escaped observation.

This lesion may perhaps assume practical importance in view of the light it may throw upon experimental gastric ulcer.

---

[a] Manuscript submitted for publication July 26, 1906.

The experimental production of true gastric ulcer, simulating the so-called peptic or round ulcer in man, has met with little success. The following table of experiments by previous investigators, taken from Turck's article upon ulcer of the stomach in the Journal of the American Medical Association, volume 46, June 9, 1906, page 1753, gives in condensed form the method used to produce experimental gastric ulcer and results obtained:

TABLES OF EXPERIMENTS BY PREVIOUS INVESTIGATORS.

A. Mechanical and physical injury.

		Result.
Ritter.	Violent bruises.	
Decker.	Heat	Ulcer.
Matthes.	Trauma (with 5 per cent HCl)	Ulcer.
Schmidt.	Trauma (with HCl).	
Körte.	Pinching stomach with clamps.	Ulcer.

B. Chemical.

Roth.	Crystals of nitrate of silver introduced into stomach	Ulcer.

1. *HCl as a necessary factor.*

Riegel.	HCl necessary.	
Matthes.	Trauma (without HCl)	Negative.
	Trauma (with 5 per cent HCl)	Ulcer.
Schmidt.	HCl and trauma.	
Ewald.	HCl essential factor.	

2. *Contra HCl.*

Pawlow.	Hyperacidity a consequence.	
Du Misinl.	Superacidity without significance.	
Ageron.	HCl may be persistently absent.	
Kavetsky.	Synchronous ulcer of the stomach and bladder.	

C. General dysemia.

Virchow.	Anemia and chlorosis.	
Quincke and Daetwyler.	Anemia by producing gradual hemorrhage and local trauma	Ulcer, which healed with difficulty.
Silbermann.	Hemoglobinemia	Ulcer.
Futterer.	Hemoglobinemia	Ulcer.
Cohnheim.	Hemoglobinemia by mechanical injury and injection of laked blood	Ulcers.

D. Disturbance of local circulation.

Virchow.	Embolism, thrombi, aneurism, or varicose veins.	
Klebs and Welti.	Thrombi.	
Panum.	Injection of emulsion of wax into femoral vein	Gastric infarcts, ulcer.
Talma.	Ligation of esophagus and pylorus	Ulcer.
Rindfleisch.	Venous stasis; prolonged ischemia.	
Axel Key.	Prolonged ischemia, due to contraction of gastric muscle	No experiments.
Müller.	Tied portal vein	Ulcer.

E. Injuries to nerves and nerve centers.

Schiff-Ebstein.	Intersection of thalami and peduncles	Ulcer.
Brown-Sequard.	Anterior corpora quadrigemina	Ulcer.
Vidova.	Injection of alcohol into vagus	Negative.
Vidova.	Section of the sympathetic	Ulcer.
Yzeren.	Section of the sympathetic with section of vagi below the diaphragm	Ulcer.
Saitta.	Section of the sympathetic with section of vagi below the diaphragm	Ulcer.
Ophüls.	Section of the sympathetic with section of the vagi below the diaphragm	Ulcer.
Koch and Ewald.	Section of cord and 5 per cent HCl in stomach	Ulcer.

F. Local infection.

Cohnheim.	Injection. Infected suspensions of lead chromate	Erosions and ulcers.
Böttcher.	Infection as cause of ulcer	Opposed by Körte.
Nauwerck.	Infection observed at edge of ulcer.	

Turck, in following the subject further has succeeded in producing ulcers of the stomach and duodenum in dogs by feeding *B. coli communis* for a variable length of time and as a result of his work considers that we now have, for the first time, a firm basis by which to solve the final or underlying etiology of gastric ulcer.

In connection with the lesion in guinea pigs which we describe it is interesting to note that Turck[a] attempted the production of gastric ulcer in dogs by the injection of diphtheria toxine into the stomach wall. This, it seemed, did not produce an ulcer, but pinhead hemorrhagic foci in the duodenum or local necroses near the pylorus. When the diphtheria toxine was injected into the mesenteric vessels, Turck also failed to produce ulcer, but obtained necroses in two weeks in the duodenum and near the pylorus.

We find that gastric ulcer may be produced in the guinea pig by the subcutaneous inoculation of diphtheria toxine given in sufficient quantity to cause the acute death of the animal. It follows injections of a minimal lethal dose, as well as the injection of the toxine-antitoxin mixture (L+ dose) used in measuring the strength of diphtheria antitoxin. When the toxine is completely neutralized by the antitoxin, as in the case of the L^0 mixture, and the animal afterwards killed, the stomach shows no lesion.

The lesion is caused by infection with the diphtheria bacillus as well as by injections of the diphtheria toxine. We injected a number of guinea pigs with a young agar culture of *B. diphtheriæ* well washed to free it of toxine. The animals sickened and died in from four to six days. The autopsy findings, so far as the stomach was concerned, were precisely similar to those seen in animals dead of toxine injections.

---

[a] Chicago Pathological Society: Transactions, April, 1903.

So far as we know a similar condition of the stomach in human diphtheria has not been observed.

The lesion in the stomach of the guinea pig consists of a sharply defined area of congestion, hemorrhage, or ulceration, near or at the pyloric extremity. Occasionally the lesion extends half an inch into the duodenum and in a few instances the duodenum itself shows slight injection or congestion, although the stomach remains normal.

The first stage consists principally of a congestion, always in the neighborhood of the pylorus. This is best seen in guinea pigs dying in about twenty-four hours as a result of overpowering doses of diphtheria toxine. When the guinea pig dies later, for instance on the second, third, or fourth day, the lesion is more advanced and consists of hemorrhages into the mucosa followed by destruction of tissue sometimes extending into the muscularis, but in no case has perforation been observed.

It it interesting to know whether guinea pigs that received less than a lethal dose of the diphtheria poison from which they ultimately recovered may have had this stomach lesion. In a few instances in our guinea pigs which died on the twenty-second and twenty-eighth days with paralysis we found old blood-stained areas in the mucosa of the stomach in the region of the pylorus, indicating a healed area.

The lesion is not the result of any particular diphtheria toxine, for we have found that it follows the use of all the various diphtheria toxines, some 25 in number, used in this laboratory.

There is no relation between the source of the guinea pig and the effects of the diphtheria toxine, since we have found this lesion in guinea pigs obtained from four outside sources as well as in those bred in the laboratory.

We have examined a number of healthy guinea pigs from our stock in order to assure ourselves that no other cause than the diphtheria toxine could account for the appearances that we find in the stomachs of guinea pigs.

It will be seen from the following table that when guinea pigs are given an injection of diphtheria toxine sufficient to kill them within twenty-four hours, about half the animals show this lesion. When they die on the third or fourth day, a very much larger proportion of the animals show the lesion. About 75 per cent of the guinea pigs dying between the fourth and fifth days show this lesion, but in guinea pigs dying later than the tenth day the relative number of animals showing this stomach lesion gradually diminishes. Guinea pigs dying of late paralysis practically never showed the acute stomach lesion.

The frequency with which guinea pigs, dead of diphtheria toxine, show the stomach lesion.

Time of death.	Condition.	Number.	Lesions.
			Per cent.
Less than 1 day	Stomach lesion	7	54
	Stomach normal	6	
Between 1 and 2 days	Stomach lesion	104	62
	Stomach normal	61	
Between 2 and 3 days	Stomach lesion	833	77
	Stomach normal	235	
Between 3 and 4 days	Stomach lesion	725	73
	Stomach normal	254	
Between 4 and 5 days	Stomach lesion	163	52
	Stomach normal	144	
Between 5 and 6 days	Stomach lesion	35	28
	Stomach normal	85	
Between 6 and 10 days	Stomach lesion	28	25
	Stomach normal	84	
More than 10 days	Stomach lesion	2	1.7
	Stomach normal	116	
Total	lesion	1,897	[a] 66
	normal	985	
		2,882	

[a] Average.

CONTROLS.

A number of comparisons were made with tetanus toxine and various chemical poisons, but in no instance was there found any similar sharply defined lesion in the pyloric extremity of the stomach.

The stomachs of guinea pigs dead of tetanus toxine appeared quite normal.

The stomachs of guinea pigs dying acutely from strong poisons, such as hydrocyanic acid, chloralcyanhydrin, etc., sometimes show acute hemorrhagic areas either of a diffuse or punctate nature, but in such cases the lesion is not confined to the pyloric end of the stomach.

For the effect of various chemical poisons upon the stomach of guinea pigs we are indebted to Dr. Reid Hunt, Chief of the Division of Pharmacology, Hygienic Laboratory, for the following data upon animals used by him in other lines of research and which serve as our controls:

Hydrocyanic acid.

Five guinea pigs were given subcutaneously from 0.005 to 0.006 mg. of hydrocyanic acid per gram of guinea pig, which caused death in one hour fifteen minutes to six hours twenty-five minutes.

G. P. No. 315 (0.005 mg.). Mucosa and submucosa of anterior and posterior walls of stomach showed large acute hemorrhagic areas. Pylorus apparently normal.

G. P. No. 305 (0.0052 mg.). Stomach showed intense hemorrhage into the mucous membrane. Pylorus apparently normal.

G. P. No. 340 (0.0055 mg.). Mucous membrane of whole of stomach and submucosa intensely hemorrhagic.

G. P. No. 327 (0.006 mg.). Enormous punctate hemorrhagic areas on mucous membrane of greater curvature of stomach.

G. P. No. 365 (0.005 mg.). Mucosa of stomach slightly injected and hemorrhagic.

Chloralcyanhydrin.

Eight guinea pigs were injected subcutaneously with chloralcyanhydrin in quantities varying from 0.0052 to 0.03 mg. per gram, which caused the death of the animals in six hours to sixteen minutes. The stomach in each instance was normal, excepting in the two animals receiving the large amounts, as follows:

G. P. No. 330 (0.025 mg.). Acute hemorrhage of the posterior and anterior walls of the stomach, involving both the mucosa and the serous coats.

G. P. No. 325 (0.03 mg.). Stomach shows numerous hemorrhagic areas.

Strophanthin.

Four guinea pigs were injected subcutaneously with strophanthin in amounts varying from 0.001 to 0.005 mg. per gram, causing the death of the animals in one hour thirty-five minutes to forty-three minutes. In each case the stomach appeared normal.

Strychnine sulphate.

Ten guinea pigs were injected subcutaneously with strychnine sulphate, and others with strychnine sulphate plus alcohol. The amount of strychnine varied from 0.004 mg. to 0.005 mg. per gram; these doses caused the death of the animals in eleven minutes to four hours twenty minutes. Stomach was normal in each instance.

Nitroprussiate of soda.

Three guinea pigs were injected with amounts of nitroprussiate of soda varying from 0.015 to 0.018 mg. per gram, which caused the death of the animals in one hour fifteen minutes to two hours fifteen minutes. The stomach was normal in all three cases.

O

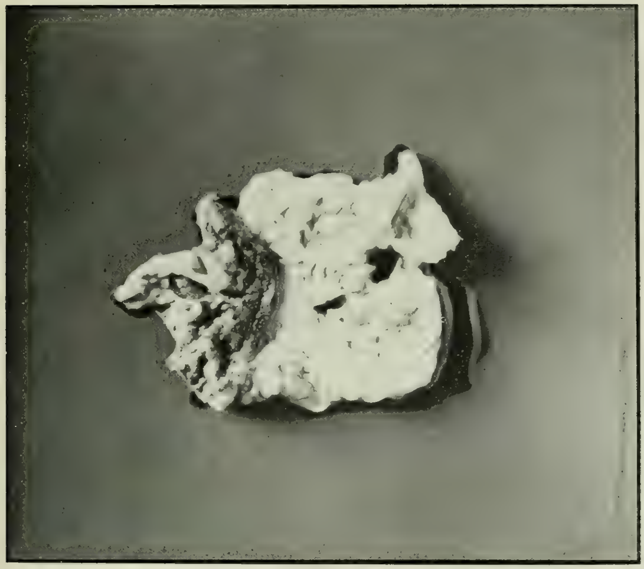

FIG. 1.—STOMACH SHOWING LESION, MILD GRADE.

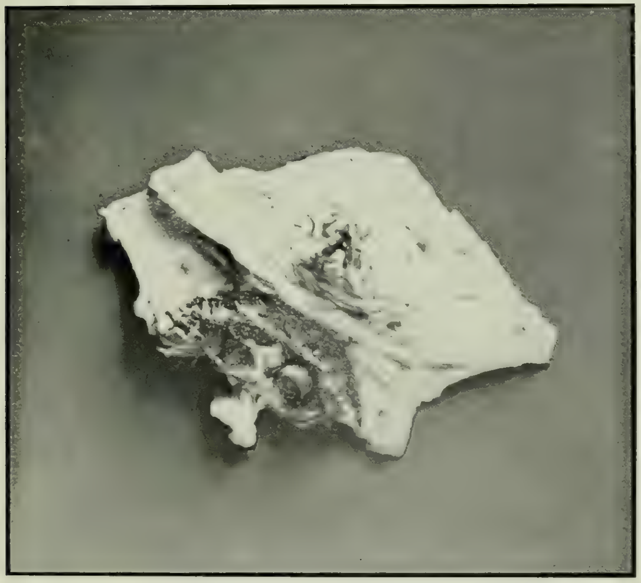

FIG. 2.—STOMACH SHOWING LESION, SEVERE GRADE.

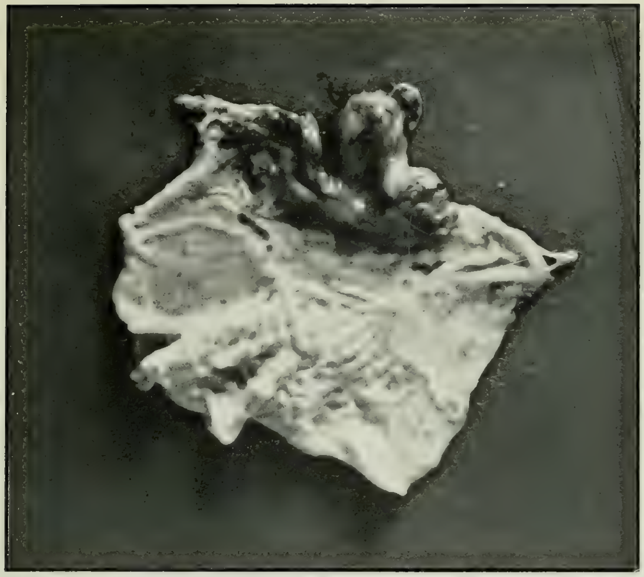

FIG. 3.—STOMACH SHOWING LESION, VERY SEVERE.

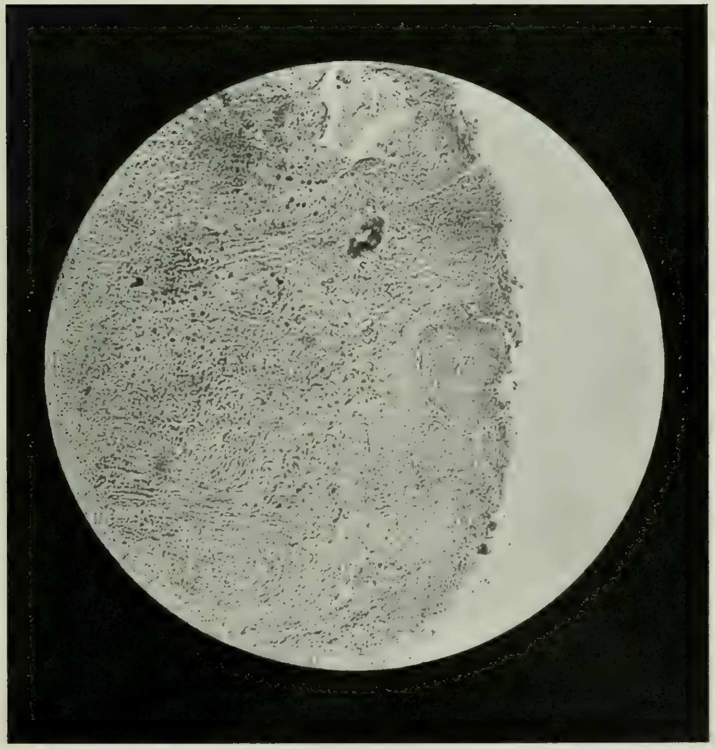

FIG. 4.—COAGULATIVE NECROSIS.

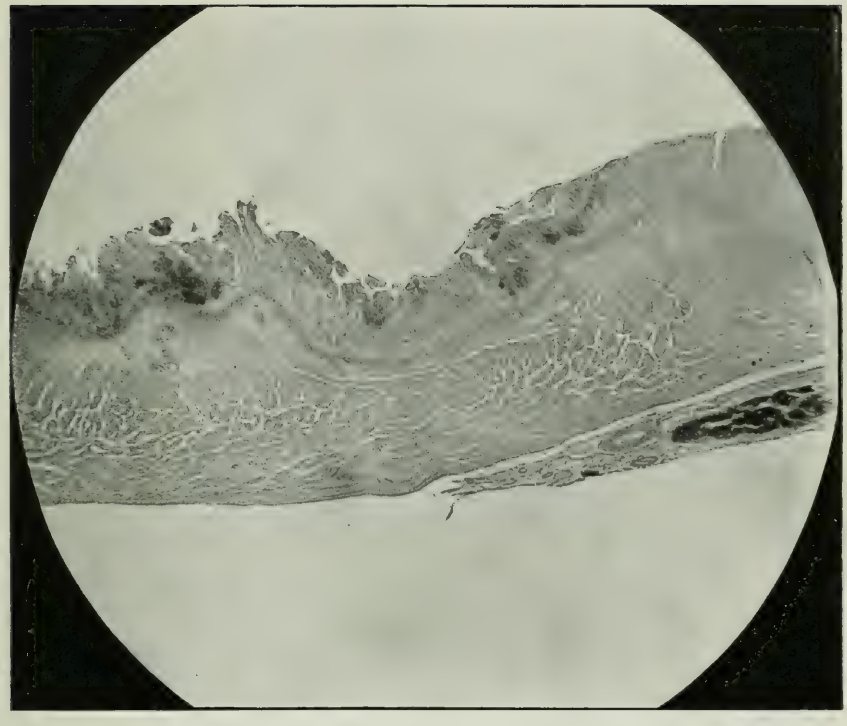

FIG. 5.—CONGESTION AND HEMORRHAGIC AREAS IN MUCOSA.

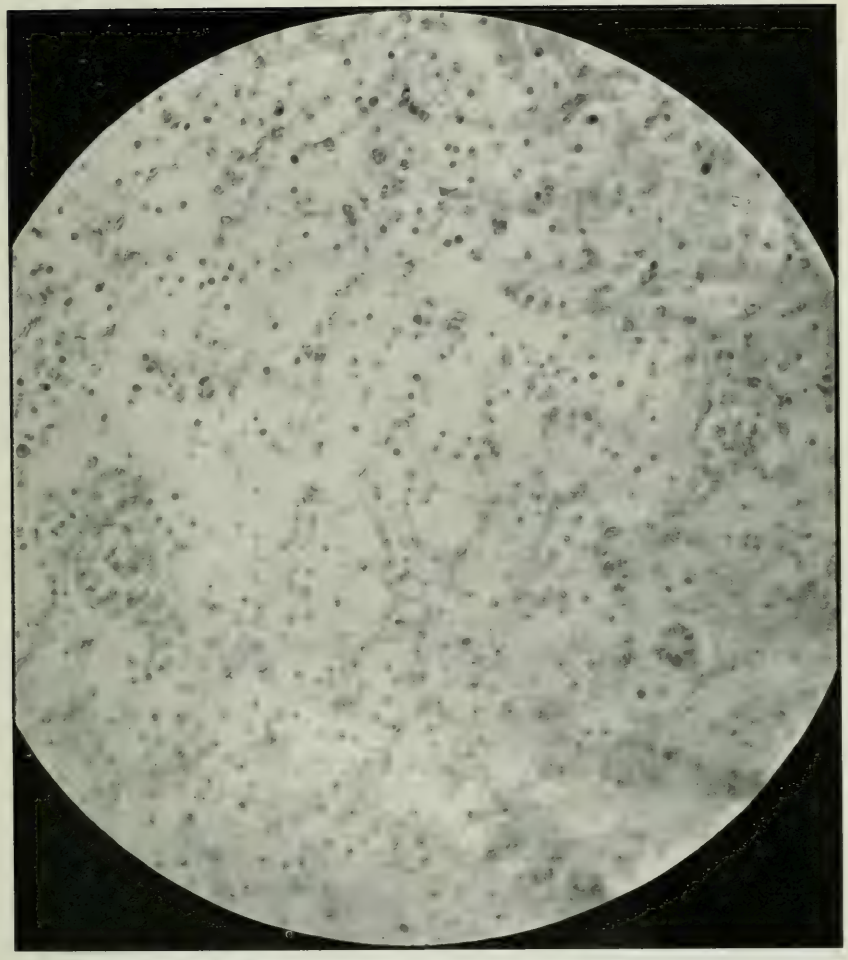

FIG. 6.—CAPILLARY CONGESTION, MUCOSA.

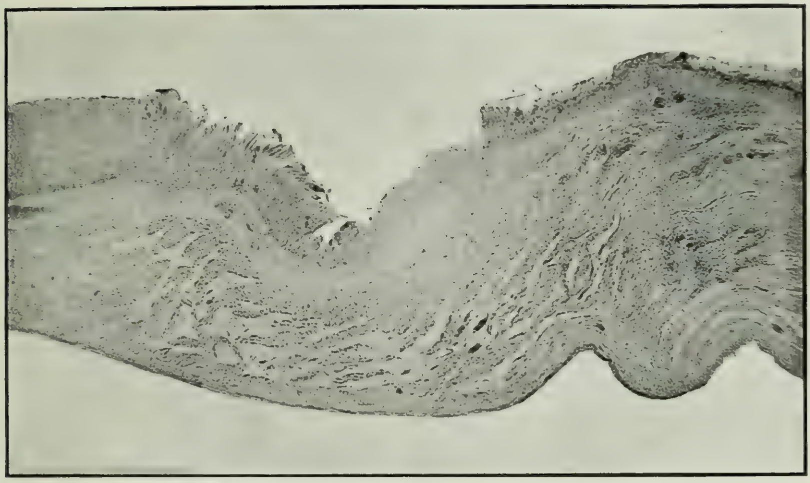

FIG. 7.—ULCER, SHOWING SHARP LINE OF DEMARCATION.

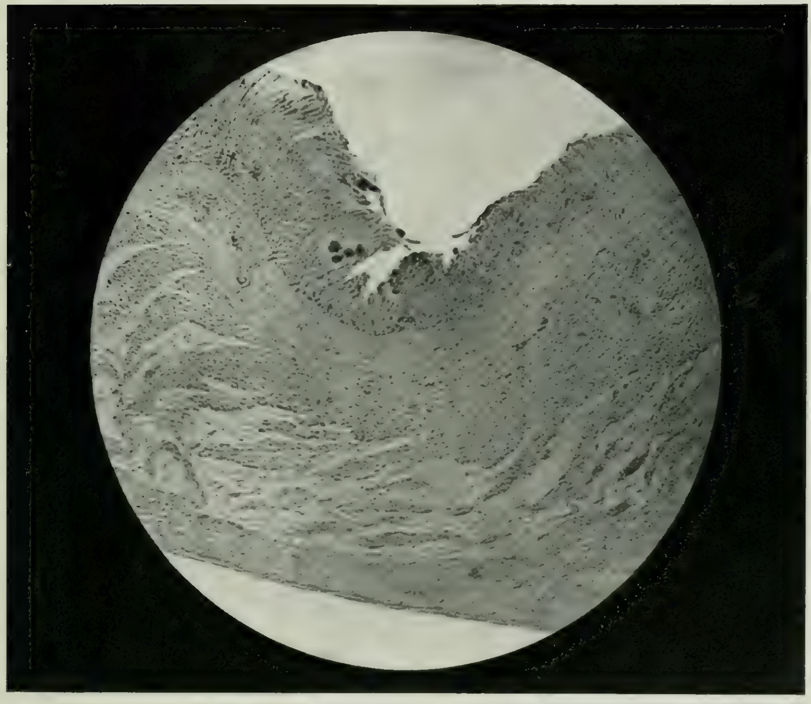

FIG. 8.—SAME AS 7. HIGHER MAGNIFICATION.

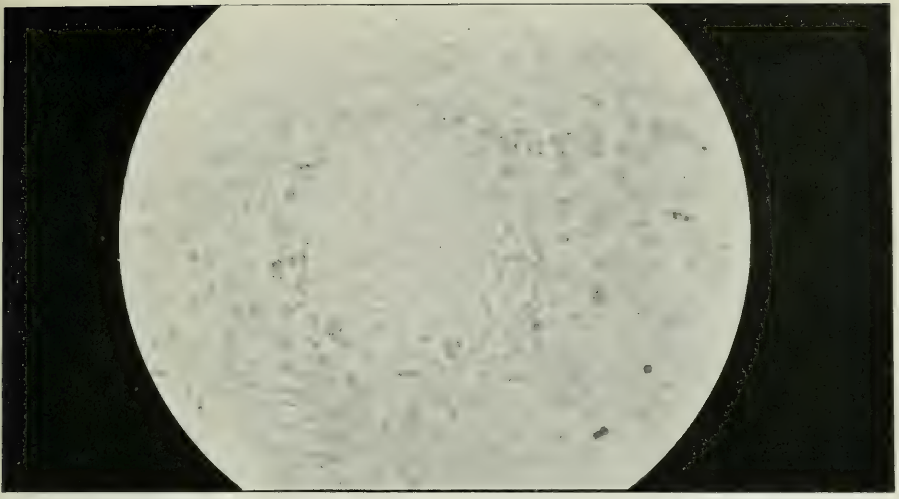

FIG. 9.—EOSINOPHILES GROUPED ABOUT PERIPHERY OF A SMALL BLOOD VESSEL.

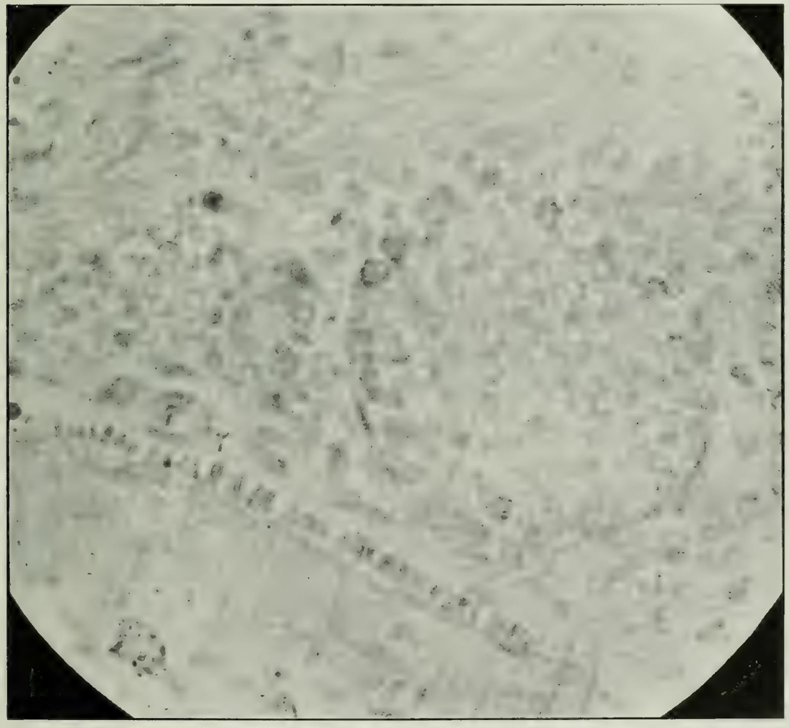

FIG. 10.—EOSINOPHILES GROUPED ABOUT PERIPHERY OF SMALL BLOOD VESSEL AT BASE OF MUCOSA.

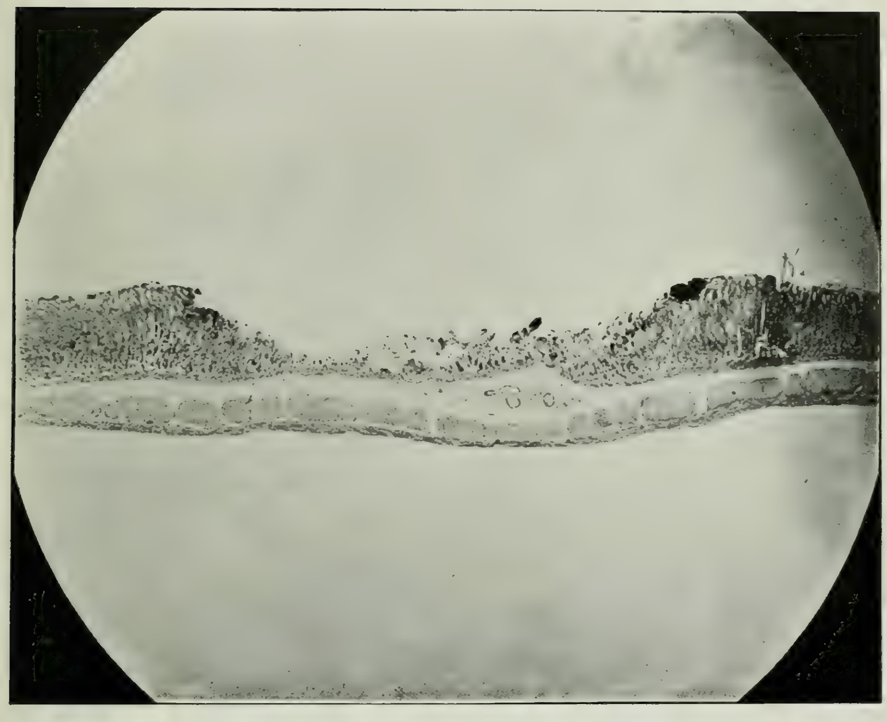

FIG. 11.—ULCER. NOTE ABSENCE OF CELLULAR INFILTRATION, INDICATING DIGESTION OF MUCOSA.

(Continued from second page of cover.)

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